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## CORRECTED VERSION

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(54) Title: NOVEL NUCLEIC ACIDS AND POLYPEPTIDES

(57) Abstract: The present invention provides novel nucleic acids, novel polypeptide sequences encoded by these nucleic acids and uses thereof.

## NOVEL NUCLEIC ACIDS AND POLYPEPTIDES

### 1. TECHNICAL FIELD

The present invention provides novel polynucleotides and proteins encoded by such
5   polynucleotides, along with uses for these polynucleotides and proteins, for example in
therapeutic, diagnostic and research methods.

### 2. BACKGROUND

Technology aimed at the discovery of protein factors (including *e.g.*, cytokines, such as
10  lymphokines, interferons, CSFs, chemokines, and interleukins) has matured rapidly over the past
decade. The now routine hybridization cloning and expression cloning techniques clone novel
polynucleotides "directly" in the sense that they rely on information directly related to the
discovered protein (*i.e.*, partial DNA/amino acid sequence of the protein in the case of
hybridization cloning; activity of the protein in the case of expression cloning). More recent
15  "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences
based on the presence of a now well-recognized secretory leader sequence motif, as well as
various PCR-based or low stringency hybridization-based cloning techniques, have advanced the
state of the art by making available large numbers of DNA/amino acid sequences for proteins
that are known to have biological activity, for example, by virtue of their secreted nature in the
20  case of leader sequence cloning, by virtue of their cell or tissue source in the case of PCR-based
techniques, or by virtue of structural similarity to other genes of known biological activity.

Identified polynucleotide and polypeptide sequences have numerous applications in, for
example, diagnostics, forensics, gene mapping; identification of mutations responsible for
genetic disorders or other traits, to assess biodiversity, and to produce many other types of data
25  and products dependent on DNA and amino acid sequences.

### 3. SUMMARY OF THE INVENTION

The compositions of the present invention include novel isolated polypeptides, novel
isolated polynucleotides encoding such polypeptides, including recombinant DNA molecules,
30  cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic
variants, antisense polynucleotide molecules, and antibodies that specifically recognize one or more
epitopes present on such polypeptides, as well as hybridomas producing such antibodies.

The compositions of the present invention additionally include vectors, including expression
vectors, containing the polynucleotides of the invention, cells genetically engineered to contain such
35  polynucleotides and cells genetically engineered to express such polynucleotides.

The present invention relates to a collection or library of at least one novel nucleic acid sequence assembled from expressed sequence tags (ESTs) isolated mainly by sequencing by hybridization (SBH), and in some cases, sequences obtained from one or more public databases. The invention relates also to the proteins encoded by such polynucleotides, along with therapeutic,

5   diagnostic and research utilities for these polynucleotides and proteins. These nucleic acid sequences are designated as SEQ ID NO: 1-30368. The polypeptides sequences are designated SEQ ID NO: 30369-60736. The nucleic acids and polypeptides are provided in the Sequence Listing. In the nucleic acids provided in the Sequence Listing, A is adenosine; C is cytosine; G is guanine; T is thymine; and N is any of the four bases. In the amino acids provided in the Sequence

10   Listing, * corresponds to the stop codon.

The nucleic acid sequences of the present invention also include, nucleic acid sequences that hybridize to the complement of SEQ ID NO: 1-30368 under stringent hybridization conditions; nucleic acid sequences which are allelic variants or species homologues of any of the nucleic acid sequences recited above, or nucleic acid sequences that encode a peptide comprising a specific

15   domain or truncation of the peptides encoded by SEQ ID NO: 1-30368. A polynucleotide comprising a nucleotide sequence having at least 90% identity to an identifying sequence of SEQ ID NO: 1-30368 or a degenerate variant or fragment thereof. The identifying sequence can be 100 base pairs in length.

The nucleic acid sequences of the present invention also include the sequence information

20   from the nucleic acid sequences of SEQ ID NO: 1-30368. The sequence information can be a segment of any one of SEQ ID NO: 1-30368 that uniquely identifies or represents the sequence information of SEQ ID NO: 1-30368.

A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or identifying information of each sequence can be provided on

25   a nucleic acid array. In one embodiment, segments of sequence information is provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array can be designed to detect full-match or mismatch to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

This invention also includes the reverse or direct complement of any of the nucleic acid

30   sequences recited above; cloning or expression vectors containing the nucleic acid sequences; and host cells or organisms transformed with these expression vectors. Nucleic acid sequences (or their reverse or direct complements) according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology, such as use as hybridization probes, use as primers for PCR, use in an array, use in computer-readable media, use in sequencing

full-length genes, use for chromosome and gene mapping, use in the recombinant production of protein, and use in the generation of anti-sense DNA or RNA, their chemical analogs and the like.

In a preferred embodiment, the nucleic acid sequences of SEQ ID NO: 1-30368 or novel segments or parts of the nucleic acids of the invention are used as primers in expression assays that are well known in the art. In a particularly preferred embodiment, the nucleic acid sequences of SEQ ID NO: 1-30368 or novel segments or parts of the nucleic acids provided herein are used in diagnostics for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The isolated polynucleotides of the invention include, but are not limited to, a polynucleotide comprising any one of the nucleotide sequences set forth in SEQ ID NO: 1-30368; a polynucleotide comprising any of the full length protein coding sequences of SEQ ID NO: 1-30368; and a polynucleotide comprising any of the nucleotide sequences of the mature protein coding sequences of SEQ ID NO: 1-30368. The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent hybridization conditions to (a) the complement of any one of the nucleotide sequences set forth in SEQ ID NO: 1-30368; (b) a nucleotide sequence encoding any one of the amino acid sequences set forth in the Sequence Listing (e.g., SEQ ID NO: 30369-60736); (c) a polynucleotide which is an allelic variant of any polynucleotides recited above; (d) a polynucleotide which encodes a species homolog (e.g. orthologs) of any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of any of the polypeptides comprising an amino acid sequence set forth in the Sequence Listing.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising any of the amino acid sequences set forth in the Sequence Listing; or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides with biological activity that are encoded by (a) any of the polynucleotides having a nucleotide sequence set forth in SEQ ID NO: 1-30368; or (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or immunologically active variants of any of the polypeptide sequences in the Sequence Listing, and "substantial equivalents" thereof (e.g., with at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% amino acid sequence identity) that preferably retain biological activity are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (e.g. host cells) of the invention.

The invention also provides compositions comprising a polypeptide of the invention. Polypeptide compositions of the invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

The invention also provides host cells transformed or transfected with a polynucleotide of the invention.

The invention also relates to methods for producing a polypeptide of the invention comprising growing a culture of the host cells of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the polypeptide from the culture or from the host cells. Preferred embodiments include those in which the protein produced by such process is a mature form of the protein.

Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers, or primers, for PCR, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of anti-sense DNA or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, *e.g., in situ* hybridization.

In other exemplary embodiments, the polynucleotides are used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide. Such antibodies, particularly monoclonal antibodies, are useful for detecting or quantitating the polypeptide in tissue. The polypeptides of the invention can also be used as molecular weight markers, and as a food supplement.

Methods are also provided for preventing, treating, or ameliorating a medical condition which comprises the step of administering to a mammalian subject a therapeutically effective amount of a composition comprising a polypeptide of the present invention and a pharmaceutically acceptable carrier.

In particular, the polypeptides and polynucleotides of the invention can be utilized, for example, in methods for the prevention and/or treatment of disorders involving aberrant protein expression or biological activity.

The present invention further relates to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample. Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders as recited herein and for the identification of subjects exhibiting a predisposition to such conditions. The invention provides

5   a method for detecting the polynucleotides of the invention in a sample, comprising contacting the sample with a compound that binds to and forms a complex with the polynucleotide of interest for a period sufficient to form the complex and under conditions sufficient to form a complex and detecting the complex such that if a complex is detected, the polynucleotide of interest is detected. The invention also provides a method for detecting the polypeptides of the

10  invention in a sample comprising contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex and detecting the formation of the complex such that if a complex is formed, the polypeptide is detected.

The invention also provides kits comprising polynucleotide probes and/or monoclonal

15  antibodies, and optionally quantitative standards, for carrying out methods of the invention. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds that modulate

20  (*i.e.*, increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited herein. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (*e.g.*, bind to) the polypeptides of the invention. The invention provides a method for identifying a

25  compound that binds to the polypeptides of the invention comprising contacting the compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and detecting the complex by detecting the reporter gene sequence expression such that if expression of the reporter gene is detected the compound that binds to a

30  polypeptide of the invention is identified.

The methods of the invention also provides methods for treatment which involve the administration of the polynucleotides or polypeptides of the invention to individuals exhibiting symptoms or tendencies. In addition, the invention encompasses methods for treating diseases or disorders as recited herein comprising administering compounds and other substances that

35  modulate the overall activity of the target gene products. Compounds and other substances can

effect such modulation either on the level of target gene/protein expression or target protein activity.

The polypeptides of the present invention and the polynucleotides encoding them are also useful for the same functions known to one of skill in the art as the polypeptides and
polynucleotides to which they have homology (set forth in the sequence listing). If no homology is set forth for a sequence, then the polypeptides and polynucleotides of the present invention are useful for a variety of applications, as described herein, including use in arrays for detection.

## 4. DETAILED DESCRIPTION OF THE INVENTION

### 4.1 DEFINITIONS

It must be noted that as used herein and in the appended claims, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise.

The term "active" refers to those forms of the polypeptide which retain the biologic and/or immunologic activities of any naturally occurring polypeptide. According to the invention, the terms "biologically active" or "biological activity" refer to a protein or peptide having structural, regulatory or biochemical functions of a naturally occurring molecule. Likewise "immunologically active" or "immunological activity" refers to the capability of the
natural, recombinant or synthetic polypeptide to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "activated cells" as used in this application are those cells which are engaged in extracellular or intracellular membrane trafficking, including the export of secretory or enzymatic molecules as part of a normal or disease process.

The terms "complementary" or "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence 5'-AGT-3' binds to the complementary sequence 3'-TCA-5'. Complementarity between two single-stranded molecules may be "partial" such that only some of the nucleic acids bind or it may be "complete" such that total complementarity exists between the single stranded molecules. The degree of
complementarity between the nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands.

The term "embryonic stem cells (ES)" refers to a cell that can give rise to many differentiated cell types in an embryo or an adult, including the germ cells. The term "germ line stem cells (GSCs)" refers to stem cells derived from primordial stem cells that provide a steady
and continuous source of germ cells for the production of gametes. The term "primordial germ

cells (PGCs)" refers to a small population of cells set aside from other cell lineages particularly from the yolk sac, mesenteries, or gonadal ridges during embryogenesis that have the potential to differentiate into germ cells and other cells. PGCs are the source from which GSCs and ES cells are derived  The PGCs, the GSCs and the ES cells are capable of self-renewal. Thus these cells

5     not only populate the germ line and give rise to a plurality of terminally differentiated cells that comprise the adult specialized organs, but are able to regenerate themselves.

The term "expression modulating fragment," EMF, means a series of nucleotides which modulates the expression of an operably linked ORF or another EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked

10    sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs are nucleic acid fragments which induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event.

The terms "nucleotide sequence" or "nucleic acid" or "polynucleotide" or

15    "oligonucleotide" are used interchangeably and refer to a heteropolymer of nucleotides or the sequence of these nucleotides. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA) or to any DNA-like or RNA-like material. In the sequences herein A is adenine, C is cytosine, T is thymine, G is guanine and N is A, C, G or T

20    (U). It is contemplated that where the polynucleotide is RNA, the T (thymine) in the sequences provided herein is substituted with U (uracil). Generally, nucleic acid segments provided by this invention may be assembled from fragments of the genome and short oligonucleotide linkers, or from a series of oligonucleotides, or from individual nucleotides, to provide a synthetic nucleic acid which is capable of being expressed in a recombinant transcriptional unit comprising

25    regulatory elements derived from a microbial or viral operon, or a eukaryotic gene.

The terms "oligonucleotide fragment" or a "polynucleotide fragment", "portion," or "segment" or "probe" or "primer" are used interchangeably and refer to a sequence of nucleotide residues which are at least about 5 nucleotides, more preferably at least about 7 nucleotides, more preferably at least about 9 nucleotides, more preferably at least about 11 nucleotides and

30    most preferably at least about 17 nucleotides. The fragment is preferably less than about 500 nucleotides, preferably less than about 200 nucleotides, more preferably less than about 100 nucleotides, more preferably less than about 50 nucleotides and most preferably less than 30 nucleotides. Preferably the probe is from about 6 nucleotides to about 200 nucleotides, preferably from about 15 to about 50 nucleotides, more preferably from about 17 to 30

35    nucleotides and most preferably from about 20 to 25 nucleotides. Preferably the fragments can

be used in polymerase chain reaction (PCR), various hybridization procedures or microarray procedures to identify or amplify identical or related parts of mRNA or DNA molecules. A fragment or segment may uniquely identify each polynucleotide sequence of the present invention. Preferably the fragment comprises a sequence substantially similar to any one of SEQ

5    ID NO: 1-30368.

Probes may, for example, be used to determine whether specific mRNA molecules are present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250). They may be labeled by nick translation, Klenow fill-in reaction, PCR, or other methods well known in the

10   art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

15   The nucleic acid sequences of the present invention also include the sequence information from the nucleic acid sequences of SEQ ID NO: 1-30368. The sequence information can be a segment of any one of SEQ ID NO: 1-30368 that uniquely identifies or represents the sequence information of that sequence of SEQ ID NO: 1-30368. One such segment can be a twenty-mer nucleic acid sequence because the probability that a twenty-mer is

20   fully matched in the human genome is 1 in 300. In the human genome, there are three billion base pairs in one set of chromosomes. Because $4^{20}$ possible twenty-mers exist, there are 300 times more twenty-mers than there are base pairs in a set of human chromosomes. Using the same analysis, the probability for a seventeen-mer to be fully matched in the human genome is approximately 1 in 5. When these segments are used in arrays for expression studies, fifteen-mer

25   segments can be used. The probability that the fifteen-mer is fully matched in the expressed sequences is also approximately one in five because expressed sequences comprise less than approximately 5% of the entire genome sequence.

Similarly, when using sequence information for detecting a single mismatch, a segment can be a twenty-five mer. The probability that the twenty-five mer would appear in a human genome

30   with a single mismatch is calculated by multiplying the probability for a full match ($1 \div 4^{25}$) times the increased probability for mismatch at each nucleotide position (3 x 25). The probability that an eighteen mer with a single mismatch can be detected in an array for expression studies is approximately one in five. The probability that a twenty-mer with a single mismatch can be detected in a human genome is approximately one in five.

The term "open reading frame," ORF, means a series of nucleotide triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

The terms "operably linked" or "operably associated" refer to functionally related nucleic acid sequences. For example, a promoter is operably associated or operably linked with a coding

5    sequence if the promoter controls the transcription of the coding sequence. While operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements *e.g.* repressor genes are not contiguously linked to the coding sequence but still control transcription/translation of the coding sequence.

The term "pluripotent" refers to the capability of a cell to differentiate into a number of

10   differentiated cell types that are present in an adult organism. A pluripotent cell is restricted in its differentiation capability in comparison to a totipotent cell.

The terms "polypeptide" or "peptide" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide or protein sequence or fragment thereof and to naturally occurring or synthetic molecules. A polypeptide "fragment," "portion," or "segment" is a stretch of amino

15   acid residues of at least about 5 amino acids, preferably at least about 7 amino acids, more preferably at least about 9 amino acids and most preferably at least about 17 or more amino acids. The peptide preferably is not greater than about 200 amino acids, more preferably less than 150 amino acids and most preferably less than 100 amino acids. Preferably the peptide is from about 5 to about 200 amino acids. To be active, any polypeptide must have sufficient

20   length to display biological and/or immunological activity.

The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

25   The term "translated protein coding portion" means a sequence which encodes for the full length protein which may include any leader sequence or any processing sequence.

The term "mature protein coding sequence" means a sequence which encodes a peptide or protein without a signal or leader sequence. The "mature protein portion" means that portion of the protein which does not include a signal or leader sequence. The peptide may have been

30   produced by processing in the cell which removes any leader/signal sequence. The mature protein portion may or may not include an initial methionine residue. The methionine residue may be removed from the protein during processing in the cell. The peptide may be produced synthetically or the protein may have been produced using a polynucleotide only encoding for the mature protein coding sequence.

The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

The term "variant"(or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using, e g., recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, i.e., conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Alternatively, where alteration of function is desired, insertions, deletions or non-conservative alterations can be engineered to produce altered polypeptides. Such alterations

can, for example, alter one or more of the biological functions or biochemical characteristics of the polypeptides of the invention. For example, such alterations may change polypeptide characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Further, such alterations can be selected so as to generate polypeptides that are better suited

5 for expression, scale up and the like in the host cells chosen for expression. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

The terms "purified" or "substantially purified" as used herein denotes that the indicated nucleic acid or polypeptide is present in the substantial absence of other biological

10 macromolecules, e.g., polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at least 95% by weight, more preferably at least 99% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

15 The term "isolated" as used herein refers to a nucleic acid or polypeptide separated from at least one other component (e.g., nucleic acid or polypeptide) present with the nucleic acid or polypeptide in its natural source. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other component normally present in a solution of the same. The terms "isolated" and "purified" do not encompass nucleic acids or

20 polypeptides present in their natural source.

The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein is derived from recombinant (e.g., microbial, insect, or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial"

25 defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, e.g., E. coli, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

30 The term "recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3)

35 appropriate transcription initiation and termination sequences. Structural units intended for use

in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an amino terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed

5    recombinant protein to provide a final product.

The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked

10   to the DNA segment or synthetic gene to be expressed. This term also means host cells which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Recombinant expression systems as defined herein will express polypeptides or proteins endogenous to the cell upon induction of the regulatory elements linked to the endogenous DNA segment or gene to be expressed. The cells

15   can be prokaryotic or eukaryotic.

The term "secreted" includes a protein that is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence when it is expressed in a suitable host cell. "Secreted" proteins include without limitation proteins secreted wholly (*e.g.*, soluble proteins) or partially (*e.g.*, receptors) from the cell in which they are

20   expressed. "Secreted" proteins also include without limitation proteins that are transported across the membrane of the endoplasmic reticulum. "Secreted" proteins are also intended to include proteins containing non-typical signal sequences (*e.g.* Interleukin-1 Beta, see Krasney, P.A. and Young, P.R. (1992) Cytokine 4(2):134 -143) and factors released from damaged cells (*e.g.* Interleukin-1 Receptor Antagonist, see Arend, W.P. et. al. (1998) Annu. Rev. Immunol.

25   16:27-55)

Where desired, an expression vector may be designed to contain a "signal or leader sequence" which will direct the polypeptide through the membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

30   The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent conditions (*i.e.*, hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1X SSC/0.1% SDS at 68°C), and moderately stringent conditions (*i.e.*, washing in 0.2X SSC/0.1% SDS at 42°C). Other exemplary hybridization conditions are

35   described herein in the examples.

In instances of hybridization of deoxyoligonucleotides, additional exemplary stringent hybridization conditions include washing in 6X SSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligonucleotides), 48°C (for 17-base oligos), 55°C (for 20-base oligonucleotides), and 60°C (for 23-base oligonucleotides).

5          As used herein, "substantially equivalent" can refer both to nucleotide and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and subject sequences. Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about 10      35% (i.e., the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.35 or less). Such a sequence is said to have 65% sequence identity to the listed sequence. In one embodiment, a substantially equivalent, e.g., mutant, sequence of the invention varies from a 15      listed sequence by no more than 30% (70% sequence identity); in a variation of this embodiment, by no more than 25% (75% sequence identity); and in a further variation of this embodiment, by no more than 20% (80% sequence identity) and in a further variation of this embodiment, by no more than 10% (90% sequence identity) and in a further variation of this embodiment, by no more that 5% (95% sequence identity). Substantially equivalent, e.g., 20      mutant, amino acid sequences according to the invention preferably have at least 80% sequence identity with a listed amino acid sequence, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% identity, more preferably at least 98% identity, and most preferably at least 99% identity.   Substantially equivalent nucleotide sequences of the invention can have lower percent sequence identities, taking into 25      account, for example, the redundancy or degeneracy of the genetic code. Preferably, nucleotide sequence has at least about 65% identity, more preferably at least about 75% identity, more preferably at least about 80% sequence identity, more preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% identity, more preferably at least about 98% sequence identity, and most preferably at least 30      about 99% sequence identity. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. For the purposes of determining equivalence, truncation of the mature sequence (e.g., via a mutation which creates a spurious stop codon) should be disregarded. Sequence identity may be determined, e.g., using the Jotun Hein method (Hein, J.

(1990) Methods Enzymol. 183:626-645). Identity between sequences can also be determined by other methods known in the art, *e.g.* by varying hybridization conditions.

The term "totipotent" refers to the capability of a cell to differentiate into all of the cell types of an adult organism.

5        The term "transformation" means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration. The term "transfection" refers to the taking up of an expression vector by a suitable host cell, whether or not any coding sequences are in fact expressed. The term "infection" refers to the introduction of nucleic acids into a suitable host cell by use of a virus or viral vector.

10      As used herein, an "uptake modulating fragment," UMF, means a series of nucleotides which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described below. The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated

15      with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence.

Each of the above terms is meant to encompass all that is described for each, unless the context dictates otherwise.

20

## 4.2 NUCLEIC ACIDS OF THE INVENTION

Nucleotide sequences of the invention are set forth in the Sequence Listing.

The isolated polynucleotides of the invention include a polynucleotide comprising the nucleotide sequences of SEQ ID NO: 1-30368; a polynucleotide encoding any one of the peptide

25      sequences of SEQ ID NO: 30369-60736; and a polynucleotide comprising the nucleotide sequence encoding the mature protein coding sequence of the polypeptides of any one of SEQ ID NO: 30369-60736. The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent conditions to (a) the complement of any of the nucleotides sequences of SEQ ID NO: 1-30368; (b) nucleotide sequences encoding any one

30      of the amino acid sequences set forth in the Sequence Listing; (c) a polynucleotide which is an allelic variant of any polynucleotide recited above; (d) a polynucleotide which encodes a species homolog of any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptides of SEQ ID NO: 30369-60736. Domains of interest may depend on the nature of the encoded polypeptide; *e.g.*, domains in

35      receptor-like polypeptides include ligand-binding, extracellular, transmembrane, or cytoplasmic

domains, or combinations thereof; domains in immunoglobulin-like proteins include the variable immunoglobulin-like domains; domains in enzyme-like polypeptides include catalytic and substrate binding domains; and domains in ligand polypeptides include receptor-binding domains.

5      The polynucleotides of the invention include naturally occurring or wholly or partially synthetic DNA, *e.g.*, cDNA and genomic DNA, and RNA, *e.g.*, mRNA. The polynucleotides may include all of the coding region of the cDNA or may represent a portion of the coding region of the cDNA.

The present invention also provides genes corresponding to the cDNA sequences disclosed 10     herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. Further 5' and 3' sequence can be obtained using methods known in the art. For example, full length cDNA or genomic DNA that 15     corresponds to any of the polynucleotides of SEQ ID NO: 1-30368 can be obtained by screening appropriate cDNA or genomic DNA libraries under suitable hybridization conditions using any of the polynucleotides of SEQ ID NO: 1-30368 or a portion thereof as a probe. Alternatively, the polynucleotides of SEQ ID NO: 1-30368 may be used as the basis for suitable primer(s) that allow identification and/or amplification of genes in appropriate genomic DNA or cDNA libraries.

20     The nucleic acid sequences of the invention can be assembled from ESTs and sequences (including cDNA and genomic sequences) obtained from one or more public databases, such as dbEST, gbpri, and UniGene. The EST sequences can provide identifying sequence information, representative fragment or segment information, or novel segment information for the full-length gene.

25     The polynucleotides of the invention also provide polynucleotides including nucleotide sequences that are substantially equivalent to the polynucleotides recited above. Polynucleotides according to the invention can have, *e.g.*, at least about 65%, at least about 70%, at least about 75%, at least about 80%, 81%, 82%, 83%, 84%, more typically at least about 85%, 86%, 87%, 88%, 89%, more typically at least about 90%, 91%, 92%, 93%, 94%, and even more typically at 30     least about 95%, 96%, 97%, 98%, 99%, sequence identity to a polynucleotide recited above.

Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequence fragments that hybridize under stringent conditions to any of the nucleotide sequences of SEQ ID NO: 1-30368, or complements thereof, which fragment is greater than about 5 nucleotides, preferably 7 nucleotides, more preferably greater than 9 nucleotides and most 35     preferably greater than 17 nucleotides. Fragments of, *e.g.* 15, 17, or 20 nucleotides or more that

are selective for (*i.e.* specifically hybridize to any one of the polynucleotides of the invention) are contemplated. Probes capable of specifically hybridizing to a polynucleotide can differentiate polynucleotide sequences of the invention from other polynucleotide sequences in the same family of genes or can differentiate human genes from genes of other species, and are

5   preferably based on unique nucleotide sequences.

The sequences falling within the scope of the present invention are not limited to these specific sequences, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO: 1-30368, a representative fragment thereof, or a nucleotide sequence at least 90% identical, preferably

10  95% identical, to SEQ ID NO: 1-30368 with a sequence from another isolate of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another codon that encodes the same amino acid is expressly contemplated.

15  The nearest neighbor or homology result for the nucleic acids of the present invention, including SEQ ID NO: 1-30368 can be obtained by searching a database using an algorithm or a program. Preferably, a BLAST which stands for Basic Local Alignment Search Tool is used to search for local sequence alignments (Altshul, S.F. J Mol. Evol. 36 290-300 (1993) and Altschul S.F. et al. J. Mol. Biol. 21:403-410 (1990)). Alternatively a FASTA version 3 search against

20  Genpept, using Fastxy algorithm.

Species homologs (or orthologs) of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

25  The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

The nucleic acid sequences of the invention are further directed to sequences which

30  encode variants of the described nucleic acids. These amino acid sequence variants may be prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. Nucleic acids encoding the amino acid sequence variants are preferably constructed by mutating the

35  polynucleotide to encode an amino acid sequence that does not occur in nature. These nucleic

acid alterations can be made at sites that differ in the nucleic acids from different species (variable positions) or in highly conserved regions (constant regions). Sites at such locations will typically be modified in series, *e.g.*, by substituting first with conservative choices (*e.g.*, hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant

5    choices (*e.g.*, hydrophobic amino acid to a charged amino acid), and then deletions or insertions may be made at the target site. Amino acid sequence deletions generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid

10   residues. Intrasequence insertions may range generally from about 1 to 10 amino residues, preferably from 1 to 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells and sequences such as FLAG or poly-histidine sequences useful for purifying the expressed protein.

In a preferred method, polynucleotides encoding the novel amino acid sequences are

15   changed via site-directed mutagenesis. This method uses oligonucleotide sequences to alter a polynucleotide to encode the desired amino acid variant, as well as sufficient adjacent nucleotides on both sides of the changed amino acid to form a stable duplex on either side of the site of being changed. In general, the techniques of site-directed mutagenesis are well known to those of skill in the art and this technique is exemplified by publications such as, Edelman et al.,

20   *DNA* 2:183 (1983). A versatile and efficient method for producing site-specific changes in a polynucleotide sequence was published by Zoller and Smith, *Nucleic Acids Res.* 10:6487-6500 (1982). PCR may also be used to create amino acid sequence variants of the novel nucleic acids. When small amounts of template DNA are used as starting material, primer(s) that differs slightly in sequence from the corresponding region in the template DNA can generate the desired

25   amino acid variant. PCR amplification results in a population of product DNA fragments that differ from the polynucleotide template encoding the polypeptide at the position specified by the primer. The product DNA fragments replace the corresponding region in the plasmid and this gives a polynucleotide encoding the desired amino acid variant.

A further technique for generating amino acid variants is the cassette mutagenesis

30   technique described in Wells et al., *Gene* 34:315 (1985); and other mutagenesis techniques well known in the art, such as, for example, the techniques in Sambrook et al., supra, and *Current Protocols in Molecular Biology*, Ausubel et al. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used in the practice of the invention for the cloning and expression

of these novel nucleic acids. Such DNA sequences include those which are capable of hybridizing to the appropriate novel nucleic acid sequence under stringent conditions.

Polynucleotides encoding preferred polypeptide truncations of the invention can be used to generate polynucleotides encoding chimeric or fusion proteins comprising one or more
5    domains of the invention and heterologous protein sequences.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above. The polynucleotide can be DNA (genomic, cDNA, amplified, or synthetic) or RNA. Methods and algorithms for obtaining such polynucleotides are well known to those of skill in the art and can include, for example, methods for determining hybridization
10   conditions that can routinely isolate polynucleotides of the desired sequence identities.

In accordance with the invention, polynucleotide sequences comprising the mature protein coding sequences corresponding to any one of SEQ ID NO: 1-30368, or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of that nucleic acid, or a functional equivalent thereof, in appropriate host cells. Also
15   included are the cDNA inserts of any of the clones identified herein.

A polynucleotide according to the invention can be joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polynucleotides include an assortment of vectors, *e.g.*,
20   plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression
25   vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The present invention further provides recombinant constructs comprising a nucleic acid having any of the nucleotide sequences of SEQ ID NO: 1-30368 or a fragment thereof or any
30   other polynucleotides of the invention. In one embodiment, the recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a nucleic acid having any of the nucleotide sequences of SEQ ID NO: 1-30368 or a fragment thereof is inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for
35   example, a promoter, operably linked to the ORF. Large numbers of suitable vectors and

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promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia).

5 Eukaryotic: pWLneo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many

10 suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed

15 (transfected) with the ligated polynucleotide/expression control sequence.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine

20 kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct

25 transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the

30 periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an amino terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product. Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination

35 signals in operable reading phase with a functional promoter. The vector will comprise one or

more phenotypic selectable markers and an origin of replication to ensure maintenance of the

vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for

transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species

within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be

5      employed as a matter of choice.

As a representative but non-limiting example, useful expression vectors for bacterial use

can comprise a selectable marker and bacterial origin of replication derived from commercially

available plasmids comprising genetic elements of the well known cloning vector pBR322

(ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine

10     Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotech, Madison, WI, USA). These

pBR322 "backbone" sections are combined with an appropriate promoter and the structural

sequence to be expressed. Following transformation of a suitable host strain and growth of the

host strain to an appropriate cell density, the selected promoter is induced or derepressed by

appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an

15     additional period. Cells are typically harvested by centrifugation, disrupted by physical or

chemical means, and the resulting crude extract retained for further purification.

Polynucleotides of the invention can also be used to induce immune responses. For

example, as described in Fan et al., *Nat. Biotech.* 17:870-872 (1999), incorporated herein by

reference, nucleic acid sequences encoding a polypeptide may be used to generate antibodies

20     against the encoded polypeptide following topical administration of naked plasmid DNA or

following injection, and preferably intramuscular injection of the DNA. The nucleic acid

sequences are preferably inserted in a recombinant expression vector and may be in the form of

naked DNA.


25     **4.3 ANTISENSE**

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that

are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide

sequence of SEQ ID NO: 1-30368, or fragments, analogs or derivatives thereof. An "antisense"

nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid

30     encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA

molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid

molecules are provided that comprise a sequence complementary to at least about 10, 25, 50,

100, 250 or 500 nucleotides or an entire coding strand, or to only a portion thereof. Nucleic acid

molecules encoding fragments, homologs, derivatives and analogs of a protein of any of SEQ ID

NO: 30369-60736 or antisense nucleic acids complementary to a nucleic acid sequence of SEQ
ID NO: 1-30368 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region"
of the coding strand of a nucleotide sequence of the invention. The term "coding region" refers
5    to the region of the nucleotide sequence comprising codons which are translated into amino acid
residues. In another embodiment, the antisense nucleic acid molecule is antisense to a
"noncoding region" of the coding strand of a nucleotide sequence of the invention. The term
"noncoding region" refers to 5' and 3' sequences which flank the coding region that are not
translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

10    Given the coding strand sequences encoding a nucleic acid disclosed herein (e.g., SEQ
ID NO: 1-30368), antisense nucleic acids of the invention can be designed according to the rules
of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be
complementary to the entire coding region of a mRNA, but more preferably is an oligonucleotide
that is antisense to only a portion of the coding or noncoding region of a mRNA. For example,
15    the antisense oligonucleotide can be complementary to the region surrounding the translation
start site of a mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25,
30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be
constructed using chemical synthesis or enzymatic ligation reactions using procedures known in
the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be
20    chemically synthesized using naturally occurring nucleotides or variously modified nucleotides
designed to increase the biological stability of the molecules or to increase the physical stability
of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate
derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid
25    include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine,
4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-
2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine,
inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,
2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine,
30    7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil,
beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil,
2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil,
queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil,
uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil,
35    3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the

antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

5          The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a protein according to the invention to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of

10   an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified

15   such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the

20   control of a strong pol II or pol III promoter are preferred.

         In yet another embodiment, the antisense nucleic acid molecule of the invention is an -a n omeric nucleic acid molecule. An -a nomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual -uni ts, the strands run parallel to each other (Gaultier *et al*. (1987) *Nucleic Acids Res* 15: 6625-6641). The

25   antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al*. (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA -DNA analogue (Inoue *et al*. (1987) *FEBS Lett* 215: 327-330).

## 4.4 RIBOZYMES AND PNA MOIETIES

30           In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as a mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave a mRNA transcripts to thereby inhibit

35   translation of a mRNA. A ribozyme having specificity for a nucleic acid of the invention can be

designed based upon the nucleotide sequence of a DNA disclosed herein (*i.e.*, SEQ ID NO: 1-30368). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an mRNA of SEQ ID NO: 1-30368 (see, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071;

5      and Cech *et al.* U.S. Pat. No. 5,116,742). Alternatively, polynucleotides of the invention can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

       Alternatively, gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region (*e.g.*, promoter and/or enhancers) to form triple helical

10     structures that prevent transcription of the gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

       In various embodiments, the nucleic acids of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or

15     solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral

20     backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

       PNAs of the invention can be used in therapeutic and diagnostic applications. For

25     example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of the invention can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or

30     primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

       In another embodiment, PNAs of the invention can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug

35     delivery known in the art. For example, PNA-DNA chimeras can be generated that may

combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e.g., RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between

5    the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA

10   and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

15         In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, Proc. *Natl. Acad. Sci.* 84:648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134). In addition,

20   oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

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## 4.5 HOSTS

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection

30   methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

Knowledge of nucleic acid sequences allows for modification of cells to permit, or

35   increase, expression of endogenous polypeptide. Cells can be modified (*e.g.*, by homologous

recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the polypeptide at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the encoding sequences. See, for example, PCT International Publication

5    No. WO94/12650, PCT International Publication No. WO92/20808, and PCT International Publication No. WO91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (*e.g.*, ada, dhfr, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the coding

10   sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by

15   calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. et al., *Basic Methods in Molecular Biology* (1986)). The host cells containing one of the polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

20   Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, 293 cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can

25   be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, New

30   York (1989), the disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981). Other cell lines capable of expressing a compatible vector are, for example, the C127, monkey COS cells, Chinese Hamster Ovary

35   (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3

cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome binding sites, polyadenylation

5  site, splice donor and acceptor sites, transcriptional termination sequences, and 5´ flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or

10  more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing

15  agents.

        Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or insects or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial

20  strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

25          In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different

30  gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by

35  targeting. These sequence include polyadenylation signals, mRNA stability elements, splice

sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.,* inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

## 4.6 POLYPEPTIDES OF THE INVENTION

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising: the amino acid sequences set forth as any one of SEQ ID NO: 30369-60736 or an amino acid sequence encoded by any one of the nucleotide sequences SEQ ID NO: 1-30368 or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides preferably with biological or immunological activity that are encoded by: (a) a polynucleotide having any one of the nucleotide sequences set forth in SEQ ID NO: 1-30368 or

(b) polynucleotides encoding any one of the amino acid sequences set forth as SEQ ID NO:
30369-60736 or (c) polynucleotides that hybridize to the complement of the polynucleotides of
either (a) or (b) under stringent hybridization conditions. The invention also provides
biologically active or immunologically active variants of any of the amino acid sequences set

5    forth as SEQ ID NO: 30369-60736 or the corresponding full length or mature protein; and
"substantial equivalents" thereof (e.g., with at least about 65%, at least about 70%, at least about
75%, at least about 80%, at least about 85%, 86%, 87%, 88%, 89%, at least about 90%, 91%,
92%, 93%, 94%, typically at least about 95%, 96%, 97%, more typically at least about 98%, or
most typically at least about 99% amino acid identity) that retain biological activity.

10   Polypeptides encoded by allelic variants may have a similar, increased, or decreased activity
compared to polypeptides comprising SEQ ID NO: 30369-60736.

          Fragments of the proteins of the present invention which are capable of exhibiting
biological activity are also encompassed by the present invention. Fragments of the protein may
be in linear form or they may be cyclized using known methods, for example, as described in H.

15   U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer.
Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such
fragments may be fused to carrier molecules such as immunoglobulins for many purposes,
including increasing the valency of protein binding sites.

          The present invention also provides both full-length and mature forms (for example,

20   without a signal sequence or precursor sequence) of the disclosed proteins. The protein coding
sequence is identified in the sequence listing by translation of the disclosed nucleotide
sequences. The mature form of such protein may be obtained by expression of a full-length
polynucleotide in a suitable mammalian cell or other host cell. The sequence of the mature form
of the protein is also determinable from the amino acid sequence of the full-length form. Where

25   proteins of the present invention are membrane bound, soluble forms of the proteins are also
provided. In such forms, part or all of the regions causing the proteins to be membrane bound
are deleted so that the proteins are fully secreted from the cell in which they are expressed.

          Protein compositions of the present invention may further comprise an acceptable carrier,
such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

30         The present invention further provides isolated polypeptides encoded by the nucleic acid
fragments of the present invention or by degenerate variants of the nucleic acid fragments of the
present invention. By "degenerate variant" is intended nucleotide fragments which differ from a
nucleic acid fragment of the present invention (e.g., an ORF) by nucleotide sequence but, due to
the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic

35   acid fragments of the present invention are the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary

5    structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. This technique is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of

10   therapeutic compounds and in immunological processes for the development of antibodies.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or

15   which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

The invention also relates to methods for producing a polypeptide comprising growing a

20   culture of host cells of the invention in a suitable culture medium, and purifying the protein from the cells or the culture in which the cells are grown. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the

25   culture, conveniently from the culture medium, or from a lysate prepared from the host cells and further purified. Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known

30   methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography. See, *e.g.*, Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in Molecular Cloning: *A Laboratory*

35   *Manual*; Ausubel et al., *Current Protocols in Molecular Biology*. Polypeptide fragments that

retain biological/immunological activity include fragments comprising greater than about 100 amino acids, or greater than about 200 amino acids, and fragments that encode specific protein domains.

The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for *e.g.*, small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the peptides of the invention or molecules capable of binding to the peptides may be complexed with toxins, *e.g.*, ricin or cholera, or with other compounds that are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for SEQ ID NO: 30369-60736.

The protein of the invention may also be expressed as a product of transgenic animals, *e.g.*, as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequence can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, *e.g.*, U.S. Pat. No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein. Regions of the protein that are important for the protein function can be determined by various methods known in the art including the alanine-scanning method which involved systematic substitution of single or strings of amino acids with alanine, followed by testing the resulting alanine-containing variant for biological activity. This type of analysis determines the importance of the substituted amino acid(s) in biological activity. Regions of the protein that are important for protein function may be determined by the eMATRIX program.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and are useful for screening or other immunological

methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing

5    an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBat™ kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present

10    invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification

15    of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl™ or Cibacrom blue 3GA Sepharose™; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

20    Alternatively, the protein of the invention may also be expressed in a form that will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX), or as a His-tag. Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and Invitrogen,

25    respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("FLAG®") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP- HPLC) steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other

30    aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The polypeptides of the invention include analogs (variants). This embraces fragments, as well as peptides in which one or more amino acids has been deleted, inserted, or substituted. Also, analogs of the polypeptides of the invention embrace fusions of the polypeptides or modifications of the polypeptides of the invention, wherein the polypeptide or analog is fused to

5      another moiety or moieties, e.g., targeting moiety or another therapeutic agent. Such analogs may exhibit improved properties such as activity and/or stability. Examples of moieties which may be fused to the polypeptide or an analog include, for example, targeting moieties which provide for the delivery of polypeptide to pancreatic cells, e.g., antibodies to pancreatic cells, antibodies to immune cells such as T-cells, monocytes, dendritic cells, granulocytes, etc., as well

10     as receptor and ligands expressed on pancreatic or immune cells. Other moieties which may be fused to the polypeptide include therapeutic agents which are used for treatment, for example, immunosuppressive drugs such as cyclosporin, SK506, azathioprine, CD3 antibodies and steroids. Also, polypeptides may be fused to immune modulators, and other cytokines such as alpha or beta interferon.

15

## 4.6.1 DETERMINING POLYPEPTIDE AND POLYNUCLEOTIDE IDENTITY AND SIMILARITY

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in computer

20     programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, FASTA (Altschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990), PSI-BLAST (Altschul S.F. et al., Nucleic Acids Res. vol. 25, pp. 3389-3402, herein incorporated by reference), eMatrix software (Wu et al., J. Comp.

25     Biol., Vol. 6, pp. 219-235 (1999), herein incorporated by reference), eMotif software (Nevill-Manning et al, ISMB-97, Vol. 4, pp. 202-209, herein incorporated by reference), pFam software (Sonnhammer et al., Nucleic Acids Res., Vol. 26(1), pp. 320-322 (1998), herein incorporated by reference) and the Kyte-Doolittle hydrophobocity prediction algorithm (J. Mol Biol, 157, pp. 105-31 (1982), incorporated herein by reference). The BLAST programs are publicly available

30     from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990).

## 4.7 CHIMERIC AND FUSION PROTEINS

The invention also provides chimeric or fusion proteins. As used herein, a "chimeric

35     protein" or "fusion protein" comprises a polypeptide of the invention operatively linked to

another polypeptide. Within a fusion protein the polypeptide according to the invention can correspond to all or a portion of a protein according to the invention. In one embodiment, a fusion protein comprises at least one biologically active portion of a protein according to the invention. In another embodiment, a fusion protein comprises at least two biologically active portions of a protein according to the invention. Within the fusion protein, the term "operatively linked" is intended to indicate that the polypeptide according to the invention and the other polypeptide are fused in-frame to each other. The polypeptide can be fused to the N-terminus or C-terminus.

For example, in one embodiment a fusion protein comprises a polypeptide according to the invention operably linked to the extracellular domain of a second protein.

In another embodiment, the fusion protein is a GST-fusion protein in which the polypeptide sequences of the invention are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences.

In another embodiment, the fusion protein is an immunoglobulin fusion protein in which the polypeptide sequences according to the invention comprises one or more domains are fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a ligand and a protein of the invention on the surface of a cell, to thereby suppress signal transduction *in vivo*. The immunoglobulin fusion proteins can be used to affect the bioavailability of a cognate ligand. Inhibition of the ligand/protein interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, *e,g.*, cancer as well as modulating (*e.g.*, promoting or inhibiting) cell survival. Moreover, the immunoglobulin fusion proteins of the invention can be used as immunogens to produce antibodies in a subject, to purify ligands, and in screening assays to identify molecules that inhibit the interaction of a polypeptide of the invention with a ligand.

A chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for

example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley &

Sons, 1992). Moreover, many expression vectors are commercially available that already encode

a fusion moiety (*e.g.*, a GST polypeptide). A nucleic acid encoding a polypeptide of the

invention can be cloned into such an expression vector such that the fusion moiety is linked

5    in-frame to the protein of the invention.

### 4.8 GENE THERAPY

Mutations in the polynucleotides of the invention gene may result in loss of normal

function of the encoded protein. The invention thus provides gene therapy to restore normal

10   activity of the polypeptides of the invention; or to treat disease states involving polypeptides of

the invention. Delivery of a functional gene encoding polypeptides of the invention to

appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly

viral vectors (*e.g.*, adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of

physical DNA transfer methods (*e.g.*, liposomes or chemical treatments). See, for example,

15   Anderson, Nature, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of

gene therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific

American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Introduction of any one of

the nucleotides of the present invention or a gene encoding the polypeptides of the present

invention can also be accomplished with extrachromosomal substrates (transient expression) or

20   artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of

proteins of the present invention in order to proliferate or to produce a desired effect on or

activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Alternatively, it is contemplated that in other human disease states, preventing the expression of

or inhibiting the activity of polypeptides of the invention will be useful in treating the disease

25   states. It is contemplated that antisense therapy or gene therapy could be applied to negatively

regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense

molecules to the nucleic acids of the present invention, their complements, or their translated RNA

sequences, by methods known in the art. Further, the polypeptides of the present invention can be

30   inhibited by using targeted deletion methods, or the insertion of a negative regulatory element such

as a silencer, which is tissue specific.

The present invention still further provides cells genetically engineered *in vivo* to express the

polynucleotides of the invention, wherein such polynucleotides are in operative association with a

regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in

the cell. These methods can be used to increase or decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can be modified (*e.g.,* by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (*e.g.,* ada, dhfr, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequences include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.,* inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are

added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the

5    property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial

10   xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436

15   (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

## 4.9 TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention in vivo, one or more genes provided by the invention are either over expressed or

20   inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be

25   prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and

30   PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a promoter of the polynucleotides of the invention is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even

35   replacing the homologous promoter to provide for increased protein expression. The

homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

The polynucleotides of the present invention also make possible the development, through, e.g., homologous recombination or knock out strategies, of animals that fail to express
5    polypeptides of the invention or that express a variant polypeptide. Such animals are useful as models for studying the in vivo activities of polypeptide as well as for studying modulators of the polypeptides of the invention.

In preferred methods to determine biological functions of the polypeptides of the invention in vivo, one or more genes provided by the invention are either over expressed or
10    inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be
15    prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and
20    PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of the polynucleotides of the invention promoter is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the
25    homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

## 4.10 USES AND BIOLOGICAL ACTIVITY

30    The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified herein. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA). The
35    mechanism underlying the particular condition or pathology will dictate whether the

polypeptides of the invention, the polynucleotides of the invention or modulators (activators or inhibitors) thereof would be beneficial to the subject in need of treatment. Thus, "therapeutic compositions of the invention" include compositions comprising isolated polynucleotides (including recombinant DNA molecules, cloned genes and degenerate variants thereof) or

5 polypeptides of the invention (including full length protein, mature protein and truncations or domains thereof), or compounds and other substances that modulate the overall activity of the target gene products, either at the level of target gene/protein expression or target protein activity. Such modulators include polypeptides, analogs, (variants), including fragments and fusion proteins, antibodies and other binding proteins; chemical compounds that directly or

10 indirectly activate or inhibit the polypeptides of the invention (identified, *e.g.*, via drug screening assays as described herein); antisense polynucleotides and polynucleotides suitable for triple helix formation; and in particular antibodies or other binding partners that specifically recognize one or more epitopes of the polypeptides of the invention.

The polypeptides of the present invention may likewise be involved in cellular activation

15 or in one of the other physiological pathways described herein.

### 4.10.1 RESEARCH USES AND UTILITIES

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant

20 protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic

25 disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as

30 an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of

35 the binding interaction.

The polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its

5   receptor) in biological fluids; as markers for tissues in which the corresponding polypeptide is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

10   Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch

15   and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

### 4.10.2 NUTRITIONAL USES

Polynucleotides and polypeptides of the present invention can also be used as nutritional

20   sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the polypeptide or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the

25   polypeptide or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

### 4.10.3 CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION ACTIVITY

30   A polypeptide of the present invention may exhibit activity relating to cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one

35   or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient

confirmation of cytokine activity. The activity of therapeutic compositions of the present

invention is evidenced by any one of a number of routine factor dependent cell proliferation

assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3,

MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e, CMK,

5    HUVEC, and Caco. Therapeutic compositions of the invention can be used in the following:

Assays for T-cell or thymocyte proliferation include without limitation those described

in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E.

M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3,

*In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in

10   Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol.

145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli,

et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or

thymocytes include, without limitation, those described in: Polyclonal T cell stimulation,

15   Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan

eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse

and human interleukin-γ, Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan

eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells

20   include, without limitation, those described in: Measurement of Human and Murine Interleukin 2

and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in

Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991;

deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988;

Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse

25   and human interleukin 6--Nordan, R. In Current Protocols in Immunology. J. E. Coligan eds. Vol

1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Aced. Sci.

U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--Bennett, F., Giannotti, J.,

Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp.

6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin

30   9--Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology.

J. E. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins

that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and

cytokine production) include, without limitation, those described in: Current Protocols in

35   Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober,

Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse
Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7,
Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095,
1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol.
137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.


### 4.10.4 STEM CELL GROWTH FACTOR ACTIVITY

A polypeptide of the present invention may exhibit stem cell growth factor activity and
be involved in the proliferation, differentiation and survival of pluripotent and totipotent stem
cells including primordial germ cells, embryonic stem cells, hematopoietic stem cells and/or
germ line stem cells. Administration of the polypeptide of the invention to stem cells *in vivo* or
*ex vivo* is expected to maintain and expand cell populations in a totipotential or pluripotential
state which would be useful for re-engineering damaged or diseased tissues, transplantation,
manufacture of bio-pharmaceuticals and the development of bio-sensors. The ability to produce
large quantities of human cells has important working applications for the production of human
proteins which currently must be obtained from non-human sources or donors, implantation of
cells to treat diseases such as Parkinson's, Alzheimer's and other neurodegenerative diseases;
tissues for grafting such as bone marrow, skin, cartilage, tendons, bone, muscle (including
cardiac muscle), blood vessels, cornea, neural cells, gastrointestinal cells and others; and organs
for transplantation such as kidney, liver, pancreas (including islet cells), heart and lung.

It is contemplated that multiple different exogenous growth factors and/or cytokines may
be administered in combination with the polypeptide of the invention to achieve the desired
effect, including any of the growth factors listed herein, other stem cell maintenance factors, and
specifically including stem cell factor (SCF), leukemia inhibitory factor (LIF), Flt-3 ligand (Flt-
3L), any of the interleukins, recombinant soluble IL-6 receptor fused to IL-6, macrophage
inflammatory protein 1-alpha (MIP-1-alpha), G-CSF, GM-CSF, thrombopoietin (TPO), platelet
factor 4 (PF-4), platelet-derived growth factor (PDGF), neural growth factors and basic
fibroblast growth factor (bFGF).

Since totipotent stem cells can give rise to virtually any mature cell type, expansion of
these cells in culture will facilitate the production of large quantities of mature cells. Techniques
for culturing stem cells are known in the art and administration of polypeptides of the invention,
optionally with other growth factors and/or cytokines, is expected to enhance the survival and
proliferation of the stem cell populations. This can be accomplished by direct administration of
the polypeptide of the invention to the culture medium. Alternatively, stroma cells transfected
with a polynucleotide that encodes for the polypeptide of the invention can be used as a feeder

layer for the stem cell populations in culture or in vivo. Stromal support cells for feeder layers may include embryonic bone marrow fibroblasts, bone marrow stromal cells, fetal liver cells, or cultured embryonic fibroblasts (see U.S. Patent No. 5,690,926).

5   Stem cells themselves can be transfected with a polynucleotide of the invention to induce autocrine expression of the polypeptide of the invention. This will allow for generation of undifferentiated totipotential/pluripotential stem cell lines that are useful as is or that can then be differentiated into the desired mature cell types. These stable cell lines can also serve as a source of undifferentiated totipotential/pluripotential mRNA to create cDNA libraries and templates for polymerase chain reaction experiments. These studies would allow for the isolation and
10  identification of differentially expressed genes in stem cell populations that regulate stem cell proliferation and/or maintenance.

Expansion and maintenance of totipotent stem cell populations will be useful in the treatment of many pathological conditions. For example, polypeptides of the present invention may be used to manipulate stem cells in culture to give rise to neuroepithelial cells that can be
15  used to augment or replace cells damaged by illness, autoimmune disease, accidental damage or genetic disorders. The polypeptide of the invention may be useful for inducing the proliferation of neural cells and for the regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders which involve degeneration, death or trauma to neural cells or nerve tissue. In
20  addition, the expanded stem cell populations can also be genetically altered for gene therapy purposes and to decrease host rejection of replacement tissues after grafting or implantation.

Expression of the polypeptide of the invention and its effect on stem cells can also be manipulated to achieve controlled differentiation of the stem cells into more differentiated cell types. A broadly applicable method of obtaining pure populations of a specific differentiated
25  cell type from undifferentiated stem cell populations involves the use of a cell-type specific promoter driving a selectable marker. The selectable marker allows only cells of the desired type to survive. For example, stem cells can be induced to differentiate into cardiomyocytes (Wobus et al., Differentiation, 48: 173-182, (1991); Klug et al., J. Clin. Invest., 98(1): 216-224, (1998)) or skeletal muscle cells (Browder, L. W. In: *Principles of Tissue Engineering eds.* Lanza et al.,
30  Academic Press (1997)). Alternatively, directed differentiation of stem cells can be accomplished by culturing the stem cells in the presence of a differentiation factor such as retinoic acid and an antagonist of the polypeptide of the invention which would inhibit the effects of endogenous stem cell factor activity and allow differentiation to proceed.

*In vitro* cultures of stem cells can be used to determine if the polypeptide of the invention
35  exhibits stem cell growth factor activity. Stem cells are isolated from any one of various cell

sources (including hematopoietic stem cells and embryonic stem cells) and cultured on a feeder layer, as described by Thompson et al. Proc. Natl. Acad. Sci, U.S.A., 92: 7844-7848 (1995), in the presence of the polypeptide of the invention alone or in combination with other growth factors or cytokines.  The ability of the polypeptide of the invention to induce stem cells

5    proliferation is determined by colony formation on semi-solid support *e.g.* as described by Bernstein et al., Blood, 77: 2316-2321 (1991).

### 4.10.5 HEMATOPOIESIS REGULATING ACTIVITY

A polypeptide of the present invention may be involved in regulation of hematopoiesis

10   and, consequently, in the treatment of myeloid or lymphoid cell disorders. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, *e.g.* in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with

15   irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (*i.e.*, traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or

20   treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and

25   paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (*i.e.*, in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

Therapeutic compositions of the invention can be used in the following:

30   Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in:  Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular

35   and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M. G. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al.,

5    Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I. K. and Briddell, R. A. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 1-21,

10   Wiley-Liss, Inc., New York, N.Y. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Long term culture initiating cell assay, Sutherland, H. J. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

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### 4.10.6 TISSUE GROWTH ACTIVITY

A polypeptide of the present invention also may be involved in bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as in wound healing and tissue repair and replacement, and in healing of burns, incisions and ulcers.

20   A polypeptide of the present invention which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Compositions of a polypeptide, antibody, binding partner, or other modulator of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of

25   artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A polypeptide of this invention may also be involved in attracting bone-forming cells, stimulating growth of bone-forming cells, or inducing differentiation of progenitors of

30   bone-forming cells. Treatment of osteoporosis, osteoarthritis, bone degenerative disorders, or periodontal disease, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes may also be possible using the composition of the invention.

Another category of tissue regeneration activity that may involve the polypeptide of the present invention is tendon/ligament formation. Induction of tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament

5    defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma

10   induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for

15   return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The compositions of the present invention may also be useful for proliferation of neural

20   cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a composition may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous

25   system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions that may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a

30   composition of the invention.

Compositions of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

Compositions of the present invention may also be involved in the generation or

35   regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine,

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kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring may allow normal tissue to regenerate. A polypeptide of the present invention may also exhibit angiogenic activity.

5          A composition of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

           A composition of the present invention may also useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the

10    growth of tissues described above.

           Therapeutic compositions of the invention can be used in the following:

           Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No.

15    WO91/07491 (skin, endothelium).

           Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

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## 4.10.7 IMMUNE STIMULATING OR SUPPRESSING ACTIVITY

           A polypeptide of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities. A

25    protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More

30    specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, proteins of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host

5    disease and autoimmune inflammatory eye disease. Such a protein (or antagonists thereof, including antibodies) of the present invention may also to be useful in the treatment of allergic reactions and conditions (e.g., anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dermatitis, erythema multiforme,

10   Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein (or antagonists thereof) of the present invention. The therapeutic effects of the

15   polypeptides or antagonists thereof on allergic reactions can be evaluated by in vivo animals models such as the cumulative contact enhancement test (Lastbom et al., Toxicology 125: 59-66, 1998), skin prick test (Hoffmann et al., Allergy 54: 446-54, 1999), guinea pig skin sensitization test (Vohr et al., Arch. Toxocol. 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

20   Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is

25   generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence

30   of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell

35   function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue

transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a therapeutic composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, a lack of costimulation may also be

5   sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

10          The efficacy of particular therapeutic compositions in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et

15   al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

          Blocking antigen function may also be therapeutically useful for treating autoimmune
20   diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self-tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block stimulation of T cells can be used to inhibit T cell activation and prevent production

25   of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine

30   experimental autoimmune encephalitis, systemic lupus erythematosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

          Upregulation of an antigen function (e.g., a B lymphocyte antigen function), as a means
35   of up regulating immune responses, may also be useful in therapy. Upregulation of immune

responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response may be useful in cases of viral infection, including systemic viral diseases such as influenza, the common cold, and encephalitis.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

A polypeptide of the present invention may provide the necessary stimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient mounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and $\beta_2$ microglobulin protein or an MHC class II alpha chain protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA

78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J.
Immunol. 135:1564-1572, 1985; Takai et al., I. Immunol. 137:3494-3500, 1986; Takai et al., J.
Immunol. 140:508-512, 1988; Bowman et al., J. Virology 61:1992-1998; Bertagnolli et al.,
Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which
will identify, among others, proteins that modulate T-cell dependent antibody responses and that
affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J.
Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production,
Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1
pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins
that generate predominantly Th1 and CTL responses) include, without limitation, those described
in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E.
M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3,
In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in
Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512,
1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed
by dendritic cells that activate naive T-cells) include, without limitation, those described in:
Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine
173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et
al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology
67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of
Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation
94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins
that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte
homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry
13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research
53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology
145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International
Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development
include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et

al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

### 4.10.8 ACTIVIN/INHIBIN ACTIVITY

A polypeptide of the present invention may also exhibit activin- or inhibin-related activities. A polynucleotide of the invention may encode a polypeptide exhibiting such characteristics. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a polypeptide of the present invention, alone or in heterodimers with a member of the inhibin family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the polypeptide of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as, but not limited to, cows, sheep and pigs.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods.

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

### 4.10.9 CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A polypeptide of the present invention may be involved in chemotactic or chemokinetic activity for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Chemotactic and chemokinetic receptor activation can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic compositions (*e.g.* proteins, antibodies, binding partners, or modulators of the invention) provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of

lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population.

5    Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

Therapeutic compositions of the invention can be used in the following:

Assays for chemotactic activity (which will identify proteins that induce or prevent

10   chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Marguiles, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates

15   and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

20       **4.10.10      HEMOSTATIC AND THROMBOLYTIC ACTIVITY**

A polypeptide of the invention may also be involved in hemostatis or thrombolysis or thrombosis. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Compositions may be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events

25   in treating wounds resulting from trauma, surgery or other causes. A composition of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (*e.g.*, stroke).

Therapeutic compositions of the invention can be used in the following:

30       Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

35       **4.10.11      CANCER DIAGNOSIS AND THERAPY**

Polypeptides of the invention may be involved in cancer cell generation, proliferation or metastasis. Detection of the presence or amount of polynucleotides or polypeptides of the invention may be useful for the diagnosis and/or prognosis of one or more types of cancer. For example, the presence or increased expression of a polynucleotide/polypeptide of the invention

5    may indicate a hereditary risk of cancer, a precancerous condition, or an ongoing malignancy. Conversely, a defect in the gene or absence of the polypeptide may be associated with a cancer condition. Identification of single nucleotide polymorphisms associated with cancer or a predisposition to cancer may also be useful for diagnosis or prognosis.

Cancer treatments promote tumor regression by inhibiting tumor cell proliferation,

10   inhibiting angiogenesis (growth of new blood vessels that is necessary to support tumor growth) and/or prohibiting metastasis by reducing tumor cell motility or invasiveness. Therapeutic compositions of the invention may be effective in adult and pediatric oncology including in solid phase tumors/malignancies, locally advanced tumors, human soft tissue sarcomas, metastatic cancer, including lymphatic metastases, blood cell malignancies including multiple myeloma,

15   acute and chronic leukemias, and lymphomas, head and neck cancers including mouth cancer, larynx cancer and thyroid cancer, lung cancers including small cell carcinoma and non-small cell cancers, breast cancers including small cell carcinoma and ductal carcinoma, gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer and polyps associated with colorectal neoplasia, pancreatic cancers, liver cancer, urologic cancers including

20   bladder cancer and prostate cancer, malignancies of the female genital tract including ovarian carcinoma, uterine (including endometrial) cancers, and solid tumor in the ovarian follicle, kidney cancers including renal cell carcinoma, brain cancers including intrinsic brain tumors, neuroblastoma, astrocytic brain tumors, gliomas, metastatic tumor cell invasion in the central nervous system, bone cancers including osteomas, skin cancers including malignant melanoma,

25   tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, hemangiopericytoma and Karposi's sarcoma.

Polypeptides, polynucleotides, or modulators of polypeptides of the invention (including inhibitors and stimulators of the biological activity of the polypeptide of the invention) may be administered to treat cancer. Therapeutic compositions can be administered in therapeutically

30   effective dosages alone or in combination with adjuvant cancer therapy such as surgery, chemotherapy, radiotherapy, thermotherapy, and laser therapy, and may provide a beneficial effect, e.g. reducing tumor size, slowing rate of tumor growth, inhibiting metastasis, or otherwise improving overall clinical condition, without necessarily eradicating the cancer.

The composition can also be administered in therapeutically effective amounts as a

35   portion of an anti-cancer cocktail. An anti-cancer cocktail is a mixture of the polypeptide or

modulator of the invention with one or more anti-cancer drugs in addition to a pharmaceutically acceptable carrier for delivery. The use of anti-cancer cocktails as a cancer treatment is routine. Anti-cancer drugs that are well known in the art and can be used as a treatment in combination with the polypeptide or modulator of the invention include: Actinomycin D,

5    Aminoglutethimide, Asparaginase, Bleomycin, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin (cis-DDP), Cyclophosphamide, Cytarabine HCl (Cytosine arabinoside), Dacarbazine, Dactinomycin, Daunorubicin HCl, Doxorubicin HCl, Estramustine phosphate sodium, Etoposide (V16-213), Floxuridine, 5-Fluorouracil (5-Fu), Flutamide, Hydroxyurea (hydroxycarbamide), Ifosfamide, Interferon Alpha-2a, Interferon Alpha-2b, Leuprolide acetate

10   (LHRH-releasing factor analog), Lomustine, Mechlorethamine HCl (nitrogen mustard), Melphalan, Mercaptopurine, Mesna, Methotrexate (MTX), Mitomycin, Mitoxantrone HCl, Octreotide, Plicamycin, Procarbazine HCl, Streptozocin, Tamoxifen citrate, Thioguanine, Thiotepa, Vinblastine sulfate, Vincristine sulfate, Amsacrine, Azacitidine, Hexamethylmelamine, Interleukin-2, Mitoguazone, Pentostatin, Semustine, Teniposide, and Vindesine sulfate.

15        In addition, therapeutic compositions of the invention may be used for prophylactic treatment of cancer. There are hereditary conditions and/or environmental situations (*e.g.* exposure to carcinogens) known in the art that predispose an individual to developing cancers. Under these circumstances, it may be beneficial to treat these individuals with therapeutically effective doses of the polypeptide of the invention to reduce the risk of developing cancers.

20        *In vitro* models can be used to determine the effective doses of the polypeptide of the invention as a potential cancer treatment. These *in vitro* models include proliferation assays of cultured tumor cells, growth of cultured tumor cells in soft agar (see Freshney, (1987) Culture of Animal Cells: A Manual of Basic Technique, Wily-Liss, New York, NY Ch 18 and Ch 21), tumor systems in nude mice as described in Giovanella et al., J. Natl. Can. Inst., 52: 921-30

25   (1974), mobility and invasive potential of tumor cells in Boyden Chamber assays as described in Pilkington et al., Anticancer Res., 17: 4107-9 (1997), and angiogenesis assays such as induction of vascularization of the chick chorioallantoic membrane or induction of vascular endothelial cell migration as described in Ribatta et al., Intl. J. Dev. Biol., 40: 1189-97 (1999) and Li et al., Clin. Exp. Metastasis, 17:423-9 (1999), respectively. Suitable tumor cells lines are available,

30   *e.g.* from American Type Tissue Culture Collection catalogs.


### 4.10.12        RECEPTOR/LIGAND ACTIVITY

A polypeptide of the present invention may also demonstrate activity as receptor, receptor ligand or inhibitor or agonist of receptor/ligand interactions. A polynucleotide of the

35   invention can encode a polypeptide exhibiting such characteristics. Examples of such receptors

54

and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen

5    recognition and development of cellular and humoral immune responses. Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

10        The activity of a polypeptide of the invention may, among other means, be measured by the following methods:

        Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley- Interscience (Chapter 7.28,

15   Measurement of Cellular Adhesion under static conditions 7.28.1- 7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

        By way of example, the polypeptides of the invention may be used as a receptor for a

20   ligand(s) thereby transmitting the biological activity of that ligand(s). Ligands may be identified through binding assays, affinity chromatography, dihybrid screening assays, BIAcore assays, gel overlay assays, or other methods known in the art.

        Studies characterizing drugs or proteins as agonist or antagonist or partial agonists or a partial antagonist require the use of other proteins as competing ligands. The polypeptides of the

25   present invention or ligand(s) thereof may be labeled by being coupled to radioisotopes, colorimetric molecules or toxin molecules by conventional methods. ("Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic Press, Inc. San Diego). Examples of radioisotopes include, but are not limited to, tritium and carbon-14. Examples of colorimetric molecules include, but are not limited to, fluorescent

30   molecules such as fluorescamine, or rhodamine or other colorimetric molecules. Examples of toxins include, but are not limited, to ricin.


        **4.10.13    DRUG SCREENING**

        This invention is particularly useful for screening chemical compounds by using the

35   novel polypeptides or binding fragments thereof in any of a variety of drug screening techniques.

The polypeptides or fragments employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or a fragment thereof. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between polypeptides of the invention or fragments and the agent being tested or examine the diminution in complex formation between the novel polypeptides and an appropriate cell line, which are well known in the art.

Sources for test compounds that may be screened for ability to bind to or modulate (*i.e.*, increase or decrease) the activity of polypeptides of the invention include (1) inorganic and organic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of either random or mimetic peptides, oligonucleotides or organic molecules.

Chemical libraries may be readily synthesized or purchased from a number of commercial sources, and may include structural analogs of known compounds or compounds that are identified as "hits" or "leads" via natural product screening.

The sources of natural product libraries are microorganisms (including bacteria and fungi), animals, plants or other vegetation, or marine organisms, and libraries of mixtures for screening may be created by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of the organisms themselves. Natural product libraries include polyketides, non-ribosomal peptides, and (non-naturally occurring) variants thereof. For a review, see *Science* 282:63-68 (1998).

Combinatorial libraries are composed of large numbers of peptides, oligonucleotides or organic compounds and can be readily prepared by traditional automated synthesis methods, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, *Curr. Opin. Biotechnol.* 8:701-707 (1997). For reviews and examples of peptidomimetic libraries, see Al-Obeidi et al., *Mol. Biotechnol,* 9(3):205-23 (1998); Hruby et al., *Curr Opin Chem Biol,* 1(1):114-19 (1997); Dorner et al., *Bioorg Med Chem,* 4(5):709-15 (1996) (alkylated dipeptides).

Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to bind a polypeptide of the invention. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the

art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

The binding molecules thus identified may be complexed with toxins, *e.g.*, ricin or cholera, or with other compounds that are toxic to cells such as radioisotopes. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for a polypeptide of the invention. Alternatively, the binding molecules may be complexed with imaging agents for targeting and imaging purposes.

### 4.10.14    ASSAY FOR RECEPTOR ACTIVITY

The invention also provides methods to detect specific binding of a polypeptide *e.g.* a ligand or a receptor. The art provides numerous assays particularly useful for identifying previously unknown binding partners for receptor polypeptides of the invention. For example, expression cloning using mammalian or bacterial cells, or dihybrid screening assays can be used to identify polynucleotides encoding binding partners. As another example, affinity chromatography with the appropriate immobilized polypeptide of the invention can be used to isolate polypeptides that recognize and bind polypeptides of the invention. There are a number of different libraries used for the identification of compounds, and in particular small molecules, that modulate (*i.e.*, increase or decrease) biological activity of a polypeptide of the invention. Ligands for receptor polypeptides of the invention can also be identified by adding exogenous ligands, or cocktails of ligands to two cells populations that are genetically identical except for the expression of the receptor of the invention: one cell population expresses the receptor of the invention whereas the other does not. The responses of the two cell populations to the addition of ligands(s) are then compared. Alternatively, an expression library can be co-expressed with the polypeptide of the invention in cells and assayed for an autocrine response to identify potential ligand(s). As still another example, BIAcore assays, gel overlay assays, or other methods known in the art can be used to identify binding partner polypeptides, including, (1) organic and inorganic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules.

The role of downstream intracellular signaling molecules in the signaling cascade of the polypeptide of the invention can be determined. For example, a chimeric protein in which the cytoplasmic domain of the polypeptide of the invention is fused to the extracellular portion of a protein, whose ligand has been identified, is produced in a host cell. The cell is then incubated with the ligand specific for the extracellular portion of the chimeric protein, thereby activating the chimeric receptor. Known downstream proteins involved in intracellular signaling can then

be assayed for expected modifications *i.e.* phosphorylation. Other methods known to those in the art can also be used to identify signaling molecules involved in receptor activity.

### 4.10.15    ANTI-INFLAMMATORY ACTIVITY

Compositions of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Compositions with such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation intimation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Compositions of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material. Compositions of this invention may be utilized to prevent or treat conditions such as, but not limited to, sepsis, acute pancreatitis, endotoxin shock, cytokine induced shock, rheumatoid arthritis, chronic inflammatory arthritis, pancreatic cell damage from diabetes mellitus type 1, graft versus host disease, inflammatory bowel disease, inflamation associated with pulmonary disease, other autoimmune disease or inflammatory disease, an antiproliferative agent such as for acute or chronic mylegenous leukemia or in the prevention of premature labor secondary to intrauterine infections.

### 4.10.16    LEUKEMIAS

Leukemias and related disorders may be treated or prevented by administration of a therapeutic that promotes or inhibits function of the polynucleotides and/or polypeptides of the invention. Such leukemias and related disorders include but are not limited to acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia).

### 4.10.17    NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested for efficacy of intervention with compounds that modulate the activity of the polynucleotides and/or polypeptides of the invention, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

(i)      traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;

(ii)     ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;

(iii)    infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;

(iv)     degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

(v)      lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;

(vi)     neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;

(vii)    lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

(viii)   demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human

immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, therapeutics which elicit any of the following effects may be useful according to the invention:

       (i)     increased survival time of neurons in culture;

       (ii)    increased sprouting of neurons in culture or in vivo;

       (iii)   increased production of a neuron-associated molecule in culture or *in vivo, e.g.,* choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

       (iv)   decreased symptoms of neuron dysfunction in vivo.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, *etc.*, depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

### 4.10.18    OTHER ACTIVITIES

A polypeptide of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye

color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape
(such as, for example, breast augmentation or diminution, change in bone form or shape);
effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female
subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or
elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, co-factors or other
nutritional factors or component(s); effecting behavioral characteristics, including, without
limitation, appetite, libido, stress, cognition (including cognitive disorders), depression
(including depressive disorders) and violent behaviors; providing analgesic effects or other pain
reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other
than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting
deficiencies of the enzyme and treating deficiency-related diseases; treatment of
hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such
as, for example, the ability to bind antigens or complement); and the ability to act as an antigen
in a vaccine composition to raise an immune response against such protein or another material or
entity which is cross-reactive with such protein.

### 4.10.19     IDENTIFICATION OF POLYMORPHISMS

The demonstration of polymorphisms makes possible the identification of such
polymorphisms in human subjects and the pharmacogenetic use of this information for diagnosis
and treatment. Such polymorphisms may be associated with, *e.g.*, differential predisposition or
susceptibility to various disease states (such as disorders involving inflammation or immune
response) or a differential response to drug administration, and this genetic information can be
used to tailor preventive or therapeutic treatment appropriately. For example, the existence of a
polymorphism associated with a predisposition to inflammation or autoimmune disease makes
possible the diagnosis of this condition in humans by identifying the presence of the
polymorphism.

Polymorphisms can be identified in a variety of ways known in the art which all
generally involve obtaining a sample from a patient, analyzing DNA from the sample, optionally
involving isolation or amplification of the DNA, and identifying the presence of the
polymorphism in the DNA. For example, PCR may be used to amplify an appropriate fragment
of genomic DNA which may then be sequenced. Alternatively, the DNA may be subjected to
allele-specific oligonucleotide hybridization (in which appropriate oligonucleotides are
hybridized to the DNA under conditions permitting detection of a single base mismatch) or to a
single nucleotide extension assay (in which an oligonucleotide that hybridizes immediately
adjacent to the position of the polymorphism is extended with one or more labeled nucleotides).

In addition, traditional restriction fragment length polymorphism analysis (using restriction enzymes that provide differential digestion of the genomic DNA depending on the presence or absence of the polymorphism) may be performed. Arrays with nucleotide sequences of the present invention can be used to detect polymorphisms. The array can comprise modified

5   nucleotide sequences of the present invention in order to detect the nucleotide sequences of the present invention. In the alternative, any one of the nucleotide sequences of the present invention can be placed on the array to detect changes from those sequences.

Alternatively a polymorphism resulting in a change in the amino acid sequence could also be detected by detecting a corresponding change in amino acid sequence of the protein, *e.g.*,

10  by an antibody specific to the variant sequence.

### 4.10.20       ARTHRITIS AND INFLAMMATION

The immunosuppressive effects of the compositions of the invention against rheumatoid arthritis are determined in an experimental animal model system. The experimental model

15  system is adjuvant induced arthritis in rats, and the protocol is described by J. Holoshitz, et at., 1983, Science, 219:56, or by B. Waksman et al., 1963, Int. Arch. Allergy Appl. Immunol., 23:129. Induction of the disease can be caused by a single injection, generally intradermally, of a suspension of killed Mycobacterium tuberculosis in complete Freund's adjuvant (CFA). The route of injection can vary, but rats may be injected at the base of the tail with an adjuvant

20  mixture. The polypeptide is administered in phosphate buffered solution (PBS) at a dose of about 1-5 mg/kg. The control consists of administering PBS only.

The procedure for testing the effects of the test compound would consist of intradermally injecting killed Mycobacterium tuberculosis in CFA followed by immediately administering the test compound and subsequent treatment every other day until day 24. At 14, 15, 18, 20, 22, and

25  24 days after injection of Mycobacterium CFA, an overall arthritis score may be obtained as described by J. Holoskitz above. An analysis of the data would reveal that the test compound would have a dramatic affect on the swelling of the joints as measured by a decrease of the arthritis score.

30  ### 4.11 THERAPEUTIC METHODS

The compositions (including polypeptide fragments, analogs, variants and antibodies or other binding partners or modulators including antisense polynucleotides) of the invention have numerous applications in a variety of therapeutic methods. Examples of therapeutic applications include, but are not limited to, those exemplified herein.

35

#### 4.11.1 EXAMPLE

One embodiment of the invention is the administration of an effective amount of the polypeptides or other composition of the invention to individuals affected by a disease or disorder that can be modulated by regulating the peptides of the invention. While the mode of administration is not particularly important, parenteral administration is preferred. An exemplary mode of administration is to deliver an intravenous bolus. The dosage of the polypeptides or other composition of the invention will normally be determined by the prescribing physician. It is to be expected that the dosage will vary according to the age, weight, condition and response of the individual patient. Typically, the amount of polypeptide administered per dose will be in the range of about 0.01μg/kg to 100 mg/kg of body weight, with the preferred dose being about 0.1μg/kg to 10 mg/kg of patient body weight. For parenteral administration, polypeptides of the invention will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of additives that maintain the isotonicity and stability of the polypeptide or other active ingredient. The preparation of such solutions is within the skill of the art.

#### 4.12    PHARMACEUTICAL FORMULATIONS AND ROUTES OF ADMINISTRATION

A protein or other composition of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources and including antibodies and other binding partners of the polypeptides of the invention) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may optionally contain (in addition to protein or other active ingredient and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the disease or disorder in question. These agents

include various growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), insulin-like growth factor (IGF), as well as cytokines described herein.

The pharmaceutical composition may further contain other agents which either enhance
5     the activity of the protein or other active ingredient or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein or other active ingredient of the invention, or to minimize side effects. Conversely, protein or other active ingredient of the present invention may be included in formulations of the particular clotting factor, cytokine,
10    lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent (such as IL-1Ra, IL-1 Hy1, IL-1 Hy2, anti-TNF, corticosteroids, immunosuppressive agents). A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or
15    complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

As an alternative to being included in a pharmaceutical composition of the invention including a first protein, a second protein or a therapeutic agent may be concurrently administered with the first protein (e.g., at the same time, or at differing times provided that
20    therapeutic concentrations of the combination of agents is achieved at the treatment site). Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, e.g., treatment, healing, prevention or amelioration of the
25    relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or
30    simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein or other active ingredient of the present invention is administered to a mammal having a condition to be treated. Protein or other active ingredient of the present invention may be administered in accordance with the method of the invention either alone or in
35    combination with other therapies such as treatments employing cytokines, lymphokines or other

hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein or other active ingredient of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein or other active ingredient of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

### 4.12.1 ROUTES OF ADMINISTRATION

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of protein or other active ingredient of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The polypeptides of the invention are administered by any route that delivers an effective dosage to the desired site of action. The determination of a suitable route of administration and an effective dosage for a particular indication is within the level of skill in the art. Preferably for wound treatment, one administers the therapeutic compound directly to the site. Suitable dosage ranges for the polypeptides of the invention can be extrapolated from these dosages or from similar studies in appropriate animal models. Dosages can then be adjusted as necessary by the clinician to provide maximal therapeutic benefit.

### 4.12.2 COMPOSITIONS/FORMULATIONS

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be

5 manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein or other active ingredient of the present invention is administered orally, protein or other active ingredient of the present

10 invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein or other active ingredient of the present invention, and preferably from about 25 to 90% protein or other active ingredient of the present invention. When administered in liquid

15 form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to

20 90% by weight of protein or other active ingredient of the present invention, and preferably from about 1 to 50% protein or other active ingredient of the present invention.

When a therapeutically effective amount of protein or other active ingredient of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein or other active ingredient of the present invention will be in the form of a pyrogen-free, parenterally

25 acceptable aqueous solution. The preparation of such parenterally acceptable protein or other active ingredient solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein or other active ingredient of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection,

30 Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's

35 solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate

to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained from a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use

in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a co-solvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may

be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.* polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose. Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well

5    known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established and are well known by those

10   skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein or other active ingredient stabilization may be employed.

     The pharmaceutical compositions also may comprise suitable solid or gel phase carriers

15   or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Many of the active ingredients of the invention may be provided as salts with pharmaceutically compatible counter ions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and

20   properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

     The pharmaceutical composition of the invention may be in the form of a complex of the

25   protein(s) or other active ingredient(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B-lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including

30   those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the

35   pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable

5  lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithins, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

10       The amount of protein or other active ingredient of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein or other active ingredient of the present invention with which to treat each individual patient. Initially, the

15  attending physician will administer low doses of protein or other active ingredient of the present invention and observe the patient's response. Larger doses of protein or other active ingredient of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should

20  contain about 0.01 µg to about 100 mg (preferably about 0.1 µg to about 10 mg, more preferably about 0.1 µg to about 1 mg) of protein or other active ingredient of the present invention per kg body weight. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic

25  composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein or other active ingredient of the invention which may also optionally be included in the composition as

30  described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing or other active ingredient-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally

capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above-mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, proteins or other active ingredients of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-$\alpha$ and TGF-$\beta$), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins or other active ingredients of the present invention. The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, *e.g.,* amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (*e.g.,* bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either in vivo or ex vivo into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured ex vivo in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes.

### 4.12.3 EFFECTIVE DOSAGE

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from appropriate in vitro assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that can be used to more accurately determine useful doses in humans. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the $IC_{50}$ as determined in cell culture (*i.e.,* the concentration of

the test compound which achieves a half-maximal inhibition of the protein's biological activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the $LD_{50}$ (the dose lethal to 50% of the population) and the $ED_{50}$ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between $LD_{50}$ and $ED_{50}$. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the $ED_{50}$ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, *e.g.*, Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1. Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the desired effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

An exemplary dosage regimen for polypeptides or other compositions of the invention will be in the range of about 0.01 μg/kg to 100 mg/kg of body weight daily, with the preferred dose being about 0.1 μg/kg to 25 mg/kg of patient body weight daily, varying in adults and children. Dosing may be once daily, or equivalent doses may be delivered at longer or shorter intervals.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's age and weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

#### 4.12.4 PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

#### 4.13 ANTIBODIES

Also included in the invention are antibodies to proteins, or fragments of proteins of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, $i.e.$, molecules that contain an antigen-binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, $F_{ab}$, $F_{ab'}$ and $F_{(ab')2}$ fragments, and an $F_{ab}$ expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as $IgG_1$, $IgG_2$, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated related protein of the invention may be intended to serve as an antigen, or a portion or fragment thereof, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein, (for example the amino acid sequence shown in SEQ ID NO: 30369), and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region on the surface of the protein of the invention that is located on the

surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human related protein sequence will indicate which regions of a related protein are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and

5   hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each of which is incorporated herein by reference in its entirety. Antibodies that are specific for one or more domains within an antigenic protein, or

10  derivatives, fragments, analogs or homologs thereof, are also provided herein.

   A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

   Various procedures known within the art may be used for the production of polyclonal or

15  monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

20  **5.13.1 Polyclonal Antibodies**

   For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic

25  protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an

30  adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. Additional examples of

adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques,
5    such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The
10   Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

### 5.13.2 Monoclonal Antibodies

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of
15   antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen-binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.
20       Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.
25       The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal
30   Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental
35   cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the

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culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium

5   such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal

10  Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by

15  immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Preferably, antibodies having a high degree of specificity and a high binding affinity for the target antigen

20  are isolated.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

25  The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as

30  those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are

35  then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or

myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, Nature 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

### 5.13.2 Humanized Antibodies

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

### 5.13.3 Human Antibodies

Fully human antibodies relate to antibody molecules in which essentially the entire sequences of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell

5    hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by

10   transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by

15   introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126;

20   5,633,425; 5,661,016, and in Marks et al. (Bio/Technology 10, 779-783 (1992)); Lonberg et al. (Nature 368 856-859 (1994)); Morrison ( Nature 368, 812-13 (1994)); Fishwild et al,( Nature Biotechnology 14, 845-51 (1996)); Neuberger (Nature Biotechnology 14, 826 (1996)); and Lonberg and Huszar (Intern. Rev. Immunol. 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals

25   which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast

30   artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse[TM] as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B

35   cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from

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the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

### 5.13.4 $F_{ab}$ Fragments and Single Chain Antibodies

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (see $e.g.$, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of $F_{ab}$ expression libraries (see $e.g.$, Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal $F_{ab}$ fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (ii) an $F_{ab}$ fragment generated

by reducing the disulfide bridges of an F$_{(ab')2}$ fragment; (iii) an F$_{ab}$ fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F$_v$ fragments.

### 5.13.5 Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., 1991 EMBO J., 10:3655-3659.

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')$_2$ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')$_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')$_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain ($V_H$) connected to a light-chain variable domain ($V_L$) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the $V_H$ and $V_L$ domains of one fragment are forced to pair with the complementary $V_L$ and $V_H$ domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

### 5.13.6 Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

### 5.13.7 Effector Function Engineering

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can

be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989).

### 5.13.8 Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include $^{212}$Bi, $^{131}$I, $^{131}$In, $^{90}$Y, and $^{186}$Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is in turn conjugated to a cytotoxic agent.

### 4.14 COMPUTER READABLE SEQUENCES

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention. As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing any of the nucleotide sequences SEQ ID NO: 1-30368 or a representative fragment thereof; or a nucleotide sequence at least 95% identical to any of the nucleotide sequences of SEQ ID NO: 1-30368 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) and BLAZE (Brutlag et al., Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system is used to identify open reading frames (ORFs) within a nucleic acid sequence. Such ORFs may be protein encoding fragments and may be useful in producing commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of a known sequence which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, Smith-Waterman, MacPattern (EMBL), BLASTN and BLASTA (NPOLYPEPTIDEIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems. As used herein, a "target sequence" can be any nucleic acid or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 300 amino acids, more preferably from about 30 to 100 nucleotide residues. However, it is well recognized that searches for commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences. Nucleic acid target motifs include, but are not limited

to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

## 4.15 TRIPLE HELIX FORMATION

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Polynucleotides suitable for use in these methods are preferably 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 15241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Olmno, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

## 4.16 DIAGNOSTIC ASSAYS AND KITS

The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using a nucleic acid probe or antibodies of the present invention, optionally conjugated or otherwise associated with a suitable label.

In general, methods for detecting a polynucleotide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polynucleotide of the invention is detected in the sample. Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polypeptide of the invention is detected in the sample.

In detail, such methods comprise incubating a test sample with one or more of the antibodies or one or more of the nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the nucleic acid probe or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention. Examples of such assays can be found in Chard, T., An Introduction to Radioimmunoassay and Related Techniques, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., Techniques in Immunocytochemistry, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1985). The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the probes or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound probe or antibody.

In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or probe. Types of detection reagents

include labeled nucleic acid probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily recognize that the disclosed probes and antibodies of the present invention can be readily incorporated into one of the

5    established kit formats which are well known in the art.

## 4.17 MEDICAL IMAGING

The novel polypeptides and binding partners of the invention are useful in medical imaging of sites expressing the molecules of the invention (e.g., where the polypeptide of the

10   invention is involved in the immune response, for imaging sites of inflammation or infection). See, e.g., Kunkel et al., U.S. Pat. NO. 5,413,778. Such methods involve chemical attachment of a labeling or imaging agent, administration of the labeled polypeptide to a subject in a pharmaceutically acceptable carrier, and imaging the labeled polypeptide in vivo at the target site.

15

## 4.18 SCREENING ASSAYS

Using the isolated proteins and polynucleotides of the invention, the present invention further provides methods of obtaining and identifying agents which bind to a polypeptide encoded by an ORF corresponding to any of the nucleotide sequences set forth in SEQ ID NO:

20   1-30368, or bind to a specific domain of the polypeptide encoded by the nucleic acid. In detail, said method comprises the steps of:

(a)    contacting an agent with an isolated protein encoded by an ORF of the present invention, or nucleic acid of the invention; and

(b)    determining whether the agent binds to said protein or said nucleic acid.

25   In general, therefore, such methods for identifying compounds that bind to a polynucleotide of the invention can comprise contacting a compound with a polynucleotide of the invention for a time sufficient to form a polynucleotide/compound complex, and detecting the complex, so that if a polynucleotide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

30   Likewise, in general, therefore, such methods for identifying compounds that bind to a polypeptide of the invention can comprise contacting a compound with a polypeptide of the invention for a time sufficient to form a polypeptide/compound complex, and detecting the complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Methods for identifying compounds that bind to a polypeptide of the invention can also comprise contacting a compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a receptor gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds a polypeptide of the invention is identified.

Compounds identified via such methods can include compounds which modulate the activity of a polypeptide of the invention (that is, increase or decrease its activity, relative to activity observed in the absence of the compound). Alternatively, compounds identified via such methods can include compounds which modulate the expression of a polynucleotide of the invention (that is, increase or decrease expression relative to expression levels observed in the absence of the compound). Compounds, such as compounds identified via the methods of the invention, can be tested using standard assays well known to those of skill in the art for their ability to modulate activity/expression.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like, capable of binding to a specific peptide sequence, in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W.H. Freeman, NY (1992), pp. 289-307, and Kaspczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control. One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic phosphodiester,

ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods preferably contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see

5    Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into

10   polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents that bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent. Agents which bind to a protein encoded by one of the ORFs of the

15   present invention can be formulated using known techniques to generate a pharmaceutical composition.


### 4.19  USE OF NUCLEIC ACIDS AS PROBES

Another aspect of the subject invention is to provide for polypeptide-specific nucleic acid

20   hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from any of the nucleotide sequences SEQ ID NO: 1-30368. Because the corresponding gene is only expressed in a limited number of tissues, a hybridization probe derived from any of the nucleotide sequences SEQ ID NO: 1-30368 can be used as an indicator of the presence of RNA of cell type of such a tissue

25   in a sample.

Any suitable hybridization technique can be employed, such as, for example, in situ hybridization. PCR as described in US Patents Nos. 4,683,195 and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both. The

30   probe will comprise a discrete nucleotide sequence for the detection of identical sequences or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means for producing specific hybridization probes for nucleic acids include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes

35   *in vitro* by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA

polymerase and the appropriate radioactively labeled nucleotides. The nucleotide sequences may be used to construct hybridization probes for mapping their respective genomic sequences. The nucleotide sequence provided herein may be mapped to a chromosome or specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These

5   techniques include in situ hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY.

10          Fluorescent *in situ* hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of a nucleic acid on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that

15  genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.

## 4.20    PREPARATION OF SUPPORT BOUND OLIGONUCLEOTIDES

Oligonucleotides, *i.e.*, small nucleic acid segments, may be readily prepared by, for example, directly synthesizing the oligonucleotide by chemical means, as is commonly practiced
20  using an automated oligonucleotide synthesizer.

Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or Teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, (1990) J. Clin. Microbiol. 28(6) 1469-72);
25  using UV light (Nagata *et al.,* 1985; Dahlen *et al.,* 1987; Morrissey & Collins, (1989) Mol. Cell Probes 3(2) 189-207) or by covalent binding of base modified DNA (Keller *et al.,* 1988; 1989); all references being specifically incorporated herein.

Another strategy that may be employed is the use of the strong biotin-streptavidin interaction as a linker. For example, Broude *et al.* (1994) Proc. Natl. Acad. Sci. USA 91(8) 3072-6,
30  describe the use of biotinylated probes, although these are duplex probes, that are immobilized on streptavidin-coated magnetic beads. Streptavidin-coated beads may be purchased from Dynal, Oslo. Of course, this same linking chemistry is applicable to coating any surface with streptavidin. Biotinylated probes may be purchased from various sources, such as, *e.g.*, Operon Technologies (Alameda, CA).

Nunc Laboratories (Naperville, IL) is also selling suitable material that could be used. Nunc Laboratories have developed a method by which DNA can be covalently bound to the microwell surface termed Covalink NH. CovaLink NH is a polystyrene surface grafted with secondary amino groups (>NH) that serve as bridge-heads for further covalent coupling. CovaLink Modules may be purchased from Nunc Laboratories. DNA molecules may be bound to CovaLink exclusively at the 5'-end by a phosphoramidate bond, allowing immobilization of more than 1 pmol of DNA (Rasmussen *et al.*, (1991) Anal. Biochem. 198(1) 138-42).

The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen et al., (1991). In this technology, a phosphoramidate bond is employed (Chu et al., (1983) Nucleic Acids Res. 11(8) 6513-29). This is beneficial as immobilization using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and then streptavidin used to bind the probes.

More specifically, the linkage method includes dissolving DNA in water (7.5 ng/ul) and denaturing for 10 min. at 95°C and cooling on ice for 10 min. Ice-cold 0.1 M 1-methylimidazole, pH 7.0 (1-MeIm$_7$), is then added to a final concentration of 10 mM 1-MeIm$_7$. A ss DNA solution is then dispensed into CovaLink NH strips (75 ul/well) standing on ice.

Carbodiimide 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), dissolved in 10 mM 1-MeIm$_7$, is made fresh and 25 ul added per well. The strips are incubated for 5 hours at 50°C. After incubation the strips are washed using, *e.g.*, Nunc-Immuno Wash; first the wells are washed 3 times, then they are soaked with washing solution for 5 min., and finally they are washed 3 times (where in the washing solution is 0.4 N NaOH, 0.25% SDS heated to 50°C).

It is contemplated that a further suitable method for use with the present invention is that described in PCT Patent Application WO 90/03382 (Southern & Maskos), incorporated herein by reference. This method of preparing an oligonucleotide bound to a support involves attaching a nucleoside 3'-reagent through the phosphate group by a covalent phosphodiester link to aliphatic hydroxyl groups carried by the support. The oligonucleotide is then synthesized on the supported nucleoside and protecting groups removed from the synthetic oligonucleotide chain under standard conditions that do not cleave the oligonucleotide from the support. Suitable reagents include nucleoside phosphoramidite and nucleoside hydrogen phosphonate.

An on-chip strategy for the preparation of DNA probe for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated photodeprotection may be

employed in the chemical synthesis of oligonucleotides directly on a glass surface, as described by
Fodor *et al.* (1991) Science 251(4995) 767-73, incorporated herein by reference. Probes may also
be immobilized on nylon supports as described by Van Ness *et al.* (1991) Nucleic Acids Res.
19(12) 3345-50; or linked to Teflon using the method of Duncan & Cavalier (1988) Anal. Biochem.
169(1) 104-8; all references being specifically incorporated herein.

     To link an oligonucleotide to a nylon support, as described by Van Ness *et al.* (1991),
requires activation of the nylon surface via alkylation and selective activation of the 5'-amine of
oligonucleotides with cyanuric chloride.

     One particular way to prepare support bound oligonucleotides is to utilize the
light-generated synthesis described by Pease *et al.*, (1994) PNAS USA 91(11) 5022-6, incorporated
herein by reference). These authors used current photolithographic techniques to generate arrays of
immobilized oligonucleotide probes (DNA chips). These methods, in which light is used to direct
the synthesis of oligonucleotide probes in high-density, miniaturized arrays, utilize photolabile
5'-protected *N*-acyl-deoxynucleoside phosphoramidites, surface linker chemistry and versatile
combinatorial synthesis strategies. A matrix of 256 spatially defined oligonucleotide probes may be
generated in this manner.

## 4.21    PREPARATION OF NUCLEIC ACID FRAGMENTS

     The nucleic acids may be obtained from any appropriate source, such as cDNAs, genomic
DNA, chromosomal DNA, microdissected chromosome bands, cosmid or YAC inserts, and RNA,
including mRNA without any amplification steps. For example, Sambrook *et al.* (1989) describes
three protocols for the isolation of high molecular weight DNA from mammalian cells (p.
9.14-9.23).

     DNA fragments may be prepared as clones in M13, plasmid or lambda vectors and/or
prepared directly from genomic DNA or cDNA by PCR or other amplification methods. Samples
may be prepared or dispensed in multiwell plates. About 100-1000 ng of DNA samples may be
prepared in 2-500 ml of final volume.

     The nucleic acids would then be fragmented by any of the methods known to those of skill
in the art including, for example, using restriction enzymes as described at 9.24-9.28 of Sambrook *et
al.* (1989), shearing by ultrasound and NaOH treatment.

     Low pressure shearing is also appropriate, as described by Schriefer *et al.* (1990) Nucleic
Acids Res. 18(24) 7455-6, incorporated herein by reference). In this method, DNA samples are
passed through a small French pressure cell at a variety of low to intermediate pressures. A lever
device allows controlled application of low to intermediate pressures to the cell. The results of

these studies indicate that low-pressure shearing is a useful alternative to sonic and enzymatic DNA fragmentation methods.

One particularly suitable way for fragmenting DNA is contemplated to be that using the two base recognition endonuclease, *CviJI*, described by Fitzgerald *et al.* (1992) Nucleic Acids Res. 20(14) 3753-62. These authors described an approach for the rapid fragmentation and fractionation of DNA into particular sizes that they contemplated to be suitable for shotgun cloning and sequencing.

The restriction endonuclease *CviJI* normally cleaves the recognition sequence PuGCPy between the G and C to leave blunt ends. Atypical reaction conditions, which alter the specificity of this enzyme (*CviJI\*\**), yield a quasi-random distribution of DNA fragments form the small molecule pUC19 (2688 base pairs). Fitzgerald *et al.* (1992) quantitatively evaluated the randomness of this fragmentation strategy, using a *CviJI\*\** digest of pUC19 that was size fractionated by a rapid gel filtration method and directly ligated, without end repair, to a lac Z minus M13 cloning vector. Sequence analysis of 76 clones showed that *CviJI\*\** restricts pyGCPy and PuGCPu, in addition to PuGCPy sites, and that new sequence data is accumulated at a rate consistent with random fragmentation.

As reported in the literature, advantages of this approach compared to sonication and agarose gel fractionation include: smaller amounts of DNA are required (0.2-0.5 ug instead of 2-5 ug); and fewer steps are involved (no preligation, end repair, chemical extraction, or agarose gel electrophoresis and elution are needed).

Irrespective of the manner in which the nucleic acid fragments are obtained or prepared, it is important to denature the DNA to give single stranded pieces available for hybridization. This is achieved by incubating the DNA solution for 2-5 minutes at 80-90°C. The solution is then cooled quickly to 2°C to prevent renaturation of the DNA fragments before they are contacted with the chip. Phosphate groups must also be removed from genomic DNA by methods known in the art.

### 4.22    PREPARATION OF DNA ARRAYS

Arrays may be prepared by spotting DNA samples on a support such as a nylon membrane. Spotting may be performed by using arrays of metal pins (the positions of which correspond to an array of wells in a microtiter plate) to repeated by transfer of about 20 nl of a DNA solution to a nylon membrane. By offset printing, a density of dots higher than the density of the wells is achieved. One to 25 dots may be accommodated in 1 mm$^2$, depending on the type of label used. By avoiding spotting in some preselected number of rows and columns, separate subsets (subarrays) may be formed. Samples in one subarray may be the same genomic segment of DNA (or the same gene) from different individuals, or may be different, overlapped genomic clones. Each of the

subarrays may represent replica spotting of the same samples. In one example, a selected gene segment may be amplified from 64 patients. For each patient, the amplified gene segment may be in one 96-well plate (all 96 wells containing the same sample). A plate for each of the 64 patients is prepared. By using a 96-pin device, all samples may be spotted on one 8 x 12 cm membrane.

5  Subarrays may contain 64 samples, one from each patient. Where the 96 subarrays are identical, the dot span may be 1 mm$^2$ and there may be a 1 mm space between subarrays.

Another approach is to use membranes or plates (available from NUNC, Naperville, Illinois) which may be partitioned by physical spacers e.g. a plastic grid molded over the membrane, the grid being similar to the sort of membrane applied to the bottom of multiwell plates, or hydrophobic

10  strips. A fixed physical spacer is not preferred for imaging by exposure to flat phosphor-storage screens or x-ray films.

The present invention is illustrated in the following examples. Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader

15  aspects of the present invention not be limited to the disclosure of the following examples. The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon

20  consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

### 5.0    EXAMPLES

25      ### 5.1    EXAMPLE 1
#### Novel Nucleic Acid Sequences Obtained From Various Libraries
A plurality of novel nucleic acids were obtained from cDNA libraries prepared from various human tissues and in some cases isolated from a genomic library derived from human chromosome using standard PCR, SBH sequence signature analysis and Sanger sequencing techniques. The

30  inserts of the library were amplified with PCR using primers specific for the vector sequences which flank the inserts. Clones from cDNA libraries were spotted on nylon membrane filters and screened with oligonucleotide probes (e.g., 7-mers) to obtain signature sequences. The clones were clustered into groups of similar or identical sequences. Representative clones were selected for sequencing.

In some cases, the 5' sequence of the amplified inserts was then deduced using a typical
Sanger sequencing protocol. PCR products were purified and subjected to fluorescent dye
terminator cycle sequencing. Single pass gel sequencing was done using a 377 Applied Biosystems
(ABI) sequencer to obtain the novel nucleic acid sequences. In some cases RACE (Rapid
5    Amplification of cDNA Ends) was performed to further extend the sequence in the 5' direction.


### 5.2      EXAMPLE 2
#### Novel Contigs

The novel contigs of the invention were assembled from sequences that were obtained from
10   a cDNA library by methods described in Example 1 above, and in some cases sequences obtained
from one or more public databases. The sequences for the resulting nucleic acid contigs are
designated as SEQ ID NO: 1-30368 and are provided in the attached Sequence Listing. The contigs
were assembled using an EST sequence as a seed. Then a recursive algorithm was used to extend
the seed EST into an extended assemblage, by pulling additional sequences from different databases
15   (*i.e.*, Hyseq's database containing EST sequences, dbEST version 115, gb pri 115, and UniGene
version 103, and exons from public domain genomic sequences predicted by GenScan) that belong
to this assemblage. The algorithm terminated when there was no additional sequences from the
above databases that would extend the assemblage. Further, the inclusion of component sequences
into the assemblage was based on a BLASTN hit to the extending assemblage with BLAST score
20   greater than 300 and percent identity greater than 95%.

The novel predicted polypeptides (including proteins) encoded by the novel polynucleotides
(SEQ ID NO: 1-30368) of the present invention are incorporated in the attached Sequence Listing.
A subset the predicted polypeptide sequences contain an unknown amino acid, a stop codon, a
possible nucleotide deletion or a possible nucleotide insertion. These sequences have been shown
25   in their entirety with the special characters in Table 2. Table 2 also shows the corresponding start
and stop nucleotide locations to each of SEQ ID NO: 1-30368. Table 2 also indicates the method
by which the polypeptide was predicted. Method A refers to a polypeptide obtained by using a
software program called FASTY (available from http://fasta.bioch.virginia.edu) which selects a
polypeptide based on a comparison of the translated novel polynucleotide to known polynucleotides
30   (W.R. Pearson, Methods in Enzymology, 183:63-98 (1990), herein incorporated by reference).
Method B refers to a polypeptide obtained by using a software program called GenScan for
human/vertebrate sequences (available from Stanford University, Office of Technology Licensing)
that predicts the polypeptide based on a probabilistic model of gene structure/compositional
properties (C. Burge and S. Karlin, J. Mol. Biol., 268:78-94 (1997), incorporated herein by

reference). Method C refers to a polypeptide obtained by using a Hyseq proprietary software
program that translates the novel polynucleotide and its complementary strand into six possible
amino acid sequences (forward and reverse frames) and chooses the polypeptide with the longest
open reading frame.

5        The nearest neighbor results for SEQ ID NO: 1-30368 were obtained by a BLASTP
version 2.0al 19MP-WashU search against Genpept release 121 and Geneseq release 200103
(Derwent), using BLAST algorithm. The nearest neighbor result showed the closest homologue
for SEQ ID NO: 1-30368. The nearest neighbor results for SEQ ID NO: 1-30368 are
incorporated in the attached Sequence Listing.

10       Using eMatrix software package (Stanford University, Stanford, CA) (Wu et al., J.
Comp. Biol., Vol. 6 pp. 219-235 (1999) herein incorporated by reference), all the sequences were
examined to determine whether they had identifiable signature regions. The attached Sequence
Listing provodes the results obtained by eMatrix analysis for each polypeptide as follows: the
signature region found in the indicated polypeptide sequences, the description of the signature,

15       the eMatrix p-value(s) and the position(s) of the signature within the polypeptide sequence.
Using the pFam software program (Sonnhammer et al., Nucleic Acids Res., Vol. 26(1)
pp. 320-322 (1998) herein incorporated by reference) all the polypeptide sequences were
examined for domains with homology to certain peptide domains. The attached Sequence
Listing provides the results obtained by PFAM analysis for each peptide, namely: the name of

20       the domain found, the description, the p-value and the pFam score for the identified domain
within the sequence.

Tables 1 and 2 follow. Table 1 shows the various tissue sources of SEQ ID NO: 1-30368.
Table 2 shows the start and stop nucleotides for the translated amino acid sequence for which each
assemblage encodes. Table 2 also provides a correlation between the amino acid sequences set forth

25       in the Sequence Listing, the nucleotide sequences set forth in the Sequence Listing and the SEQ ID
NO: in USSN 09/540,217

Table 1

| Tissue origin | RNA Source | Library Name | SEQ ID NOS: |
|---|---|---|---|
| adult brain | GIBCO | AB3001 | 39-41 192 197-200 315-316 540-542 576-580 608-622 635 1004 1185-1187 1273-1279 1431 1474 1721-1722 2036 2136-2137 2457 2471-2474 2513 2599-2603 2988-2989 3105-3106 3212 3276-3277 3306-3308 3352 3365 3374-3376 3433 3448-3450 3555-3558 3693 3949-3953 4067-4072 4160-4162 4558-4560 4581-4582 4612-4614 4837-4840 5483-5484 5603-5606 5700 5802 5980-5984 6135-6136 6403-6404 6452-6453 7209-7212 7447-7449 7452-7460 7536-7541 7554-7555 7622-7623 7630-7636 7660-7665 7701-7703 7771 7778-7783 7798-7801 7921-7923 7994 8010-8012 8025-8026 8145-8151 8227-8229 8415 8497-8499 8936-8938 8986-8991 9002-9004 9013-9017 9337-9338 9366-9368 9375-9376 9391-9392 9395-9396 9431-9436 9443 9475-9476 9517-9518 9522-9525 9586-9589 9603-9604 9851-9852 9854-9855 9874-9895 9905-9908 9947-9952 9969-9980 9986-9992 10025-10026 10033-10037 10167-10172 10277 10480-10482 10488-10489 10498-10503 10520-10522 10537-10538 10592-10594 10628-10630 11226-11227 11339-11344 11406-11407 11431-11432 11731-11734 12150-12151 12239 12241-12244 12555-12559 12615-12618 12785-12787 12978-12981 12984-12985 12997-12999 13567-13568 13592-13595 13606-13608 13873-13875 13999-14004 14360-14369 14650-14651 14684-14685 15013-15018 15096 15174-15181 15209-15210 15250-15251 15257 15323-15324 15548-15552 15568-15572 15576-15577 15588-15589 15699-15700 15881-15883 16438-16439 16473-16478 16496-16497 16609-16611 16686-16693 16700-16701 16727-16729 16836-16842 16934-16937 16949-16953 17455-17456 17857-17861 17958-17963 18029-18030 18136-18138 18423-18425 18516-18518 18535-18537 18624-18626 18668-18672 18719-18722 18750-18756 18790-18793 18802-18804 18836-18838 18899-18903 18919-18921 18943-18945 18947-18950 18964-18969 18989-18990 19013-19017 19045-19048 19057-19065 19142-19147 19154-19155 19224 19316-19317 19345-19349 19355-19360 19362 19370 19385-19389 19415-19417 19422-19431 19442-19444 19503 19560-19562 19566 19604-19607 19693 19709-19710 19727-19732 19736-19742 19772 19804-19808 19921-19929 19933-19938 19943-19946 19969-19981 20015-20017 20029-20043 20087-20094 20099-20102 20111-20112 20122-20127 20161-20164 20167-20171 20180-20181 20189-20194 20198-20199 20215-20218 20281-20282 20289 20321-20324 20349-20354 20361 20393-20400 20415-20417 20437-20440 20524-20535 20542-20545 20554-20558 20607-20612 20614-20615 20646-20652 20698-20707 20718-20725 20727-20732 20789-20791 20806-20812 20844-20849 20888-20889 20926 20938-20942 20999-21004 21027-21031 21062-21066 21072-21075 21137-21140 21145-21148 21153- |

| Tissue origin | RNA Source | Library Name | SEQ ID NOS: |
|---|---|---|---|
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| bone marrow | Clontech | BMD004 | 656 1048 5335-5336 8768-8770 9956-9957 12872-12881 15290-15291 18673-18677 18719-18722 19156-19158 21978 22669-22670 23046 25373 26843 27193-27200 27218 27790-27796 27989-27990 28199-28203 28286 |
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| adult cervix | BioChain | CVX001 | 30 48-49 51-52 144-145 156 180-181 201 221 255-256 301 329-330 332 395 419-420 425-427 515-517 524-525 530-531 543-547 551-552 694-695 716-720 738-740 832-836 901-904 934-935 1001-1003 1047 1185-1187 1259-1265 1273-1279 1302-1303 1424-1429 1431 1453-1454 1456-1460 1474 1483 1499-1502 1563-1564 1596 1667-1668 1705-1714 1800 1866-1872 1887-1889 1899-1900 2015-2018 2036 2062 2220-2227 2275-2276 2307 2375 2404-2406 2431 2471-2474 2516 2540-2541 2599-2603 2626-2642 2644-2651 2653-2658 2661-2673 2692-2694 2710 2802 2921-2930 2951-2955 2988-2989 3001 3090 3205-3207 3245-3246 3268-3269 3290-3303 3421-3422 3455-3456 3466 3507-3510 3512-3520 3526-3529 3531-3534 3555-3558 3661-3670 3705-3706 3763-3765 3817 3949-3953 4122-4123 4160-4162 4221-4238 4469-4470 4599-4601 4663-4665 4692-4694 4762-4763 4785-4789 4795-4809 4819-4823 4957 5084-5085 5092-5094 5109-5115 5124-5126 5133 5335-5336 5364 5483-5484 5581-5583 5654 5766-5767 5932-5936 5973-5975 5980-5984 5997-6000 6011-6021 6284 6371-6376 6403-6404 6484 6486-6489 6624-6626 6779 6877-6881 6932-6938 6980 7192-7193 7209-7212 7216-7219 7279-7282 7294-7295 7350-7352 7534-7550 7576-7577 7601-7603 7609 7640-7642 7671-7673 7678-7689 7693-7694 7701-7703 7728 7742-7743 7747-7751 7771 7778-7783 7787-7788 7794-7801 7890 7924-7925 7976 7988 8020 8025-8026 8070 8084-8085 8115-8121 8132-8141 8145-8156 8184-8186 8258-8262 8270-8276 8313-8314 8342 8347-8360 8368-8370 8383-8389 8415 8422-8425 8430-8433 8466-8476 8504-8506 8543-8550 8688 8751-8753 8758-8759 8869 8917-8933 8982-8983 8986-8991 9021 9029-9035 9037 9072-9074 9196-9200 9305 9321-9323 9337-9338 9391-9392 9427-9428 9444 9456-9458 9487-9488 9506-9509 9522-9525 9531-9534 9617-9626 9684-9687 9709 9729-9731 9763-9767 9794-9795 9815-9820 9826 9828 9854-9856 9899-9901 9916-9920 9923-9924 9958-9961 9969-9980 9989-9992 9997-10009 10017-10019 10038-10040 10175-10196 10273-10274 10277 10306 10451-10453 10484-10489 10498-10503 10508-10515 10523-10532 10539-10542 10588-10594 10873-10875 10877-10878 10886-10889 10901-10902 10979 11013-11017 11028-11031 11147-11156 11217-11219 11252-11273 11300-11303 11317 11345-11350 11423-11426 11431-11432 |

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| induced neuron cells | Stratagene | NTD001 | 188-191 289-291 576-578 617-618 716-717 1030 1034 1097-1098 1646 2540-2541 2599-2603 2675-2678 2724-2726 2871-2873 2973 3326-3331 3374-3376 3649-3653 3908-3910 3930 3949-3953 4165-4170 4486-4487 4515-4516 4581-4582 4984-4985 5053-5054 5272-5275 5335-5336 5777-5778 5802 5919-5921 5980-5984 6403-6404 6787-6790 6795-6800 7170-7172 7258-7263 7325-7343 7363 7368-7369 7557-7559 7688-7689 7693-7694 7701-7703 7745-7746 7778-7783 7906-7912 7990-7992 8187-8200 8250 8497-8499 8689-8690 8758-8759 8998-9001 9029-9030 9040-9042 9047-9048 9087 9321-9323 9559-9560 9829-9832 9909-9912 9947-9952 9993-9996 10477-10478 10494 10531-10532 10592-10594 10615-10623 10842-10867 10980-10985 11045 11228-11229 11314-11315 11405 11431-11432 11541-11543 11546 11609 11700-11707 11739-11740 11803-11808 11886-11891 11941-11944 12131-12132 12241-12244 12258-12262 12898-12899 12902-12905 12997-12999 13592-13595 13609-13612 13652-13659 14304-14305 15182-15183 15190-15191 15290-15291 15588-15589 15969-15974 16028-16029 16180-16181 16545 16619-16621 16642 17292-17296 17401-17432 17435 17455-17456 18029-18030 18097-18134 18300-18307 18400-18402 18412-18418 18691-18692 18771-18772 18796-18801 18839-18841 18846-18853 18899-18903 18989-18990 19001-19004 19012 19074-19080 19106-19118 19207-19208 19256-19257 19266-19267 19306-19307 19316-19317 19343 19355-19360 19387-19389 19447-19448 19458-19460 19488-19493 19566 19598-19601 19617-19619 19659-19661 19736-19742 19804-19808 19813 19939 19972-19980 20029-20043 20099-20102 20106-20110 20114-20119 20122-20127 20182-20188 20208-20218 20485-20490 20521-20523 20607-20612 20681 20698-20707 20827-20835 20853-20854 20871 21105-21111 21248-21271 21275-21280 21284-21294 21463-21465 21495-21500 21587-21646 21929-21935 22020-22025 22045-22046 22070-22073 22141-22143 22160 22187-22192 22195-22198 22243-22245 22358-22359 22365-22371 22381-22388 22433 22653-22654 22671-22674 22690 22916-22922 22977 23201-23202 23251 23358-23360 23420-23421 23700-23701 23798-23799 23806-23809 23890-23892 24749-24754 24928-24938 24985-24988 25029-25040 25279-25288 25376-25379 26024-26028 26205-26206 26209-26213 26266 26280 26310-26314 26327 26361-26365 26678-26680 27091 27100-27102 27269-27270 27446-27449 27522-27539 27544-27545 27729-27739 27861-27864 27896-27927 27989-27990 28315-28316 28361-28364 29117-29123 29328-29337 29343-29345 29418-29419 29426-29431 29885-29891 30167-30177 30352 30361-30368 |

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| neuronal cells | Stratagene | NTU001 | 240-250 373-374 425-427 576-578 847-848 1388-1401 1432-1435 1470 1499 1778-1779 2306 2599-2603 2944 3040-3076 3107-3108 3326-3331 3421-3422 3477-3478 3483-3484 3789-3794 3912-3915 4055-4058 4171-4172 4206-4208 4515-4516 4562-4568 4581-4582 4785-4789 5186-5189 5253-5266 5272-5275 5279-5283 5525-5526 5943-5945 6135-6136 6403-6404 7264-7266 7346-7352 7784-7786 7815-7818 8203 8227-8229 8465 8497-8499 8716-8718 8998-9004 9007-9008 9038-9039 9049-9068 9472-9474 9479-9481 9535-9537 9594-9602 9735-9738 9929-9935 9969-9980 10161-10163 10167-10196 10516-10518 10615-10623 10873-10875 10915-10918 11308-11310 11334-11335 11775-11776 11840 12150-12151 12258 12590-12592 12653-12654 12716-12719 12997-12999 13552-13555 13638-13641 13847-13849 14038-14041 14044-14045 14137-14138 14277-14280 14640-14642 14814-14815 15025-15069 15100-15109 15277-15278 15408-15411 15530-15531 15563-15564 15576-15577 15588-15589 15863-15870 16141-16143 16174-16176 16182-16189 16545 16642 16652 16836-16842 16851-16853 17284-17285 17435-17440 17451-17454 17958-17962 18029-18030 18043 18097-18134 18500-18501 18562-18576 18671-18672 18796-18801 18825-18828 18857-18880 18925-18933 18975 18993-18995 19049-19053 19153 19298-19300 19306-19307 19316-19317 19351-19354 19370 19375-19379 19395-19400 19415-19417 19432-19434 19511 19515-19521 19526-19529 19564-19566 19625 19659-19661 19683-19687 19813-19815 19855-19856 19938 19940-19942 19965-19967 19972-19980 20026-20027 20099-20102 20106-20110 20161-20164 20200-20207 20241 20289 20336-20338 20406-20407 20437-20440 20485-20490 20521-20523 20537-20541 20602-20612 20631-20634 20639-20640 20646-20648 20666-20671 20792-20797 20827-20843 20871 20897-20900 20924-20925 20927-20928 20938-20942 20957-20962 21032-21045 21208-21210 21241-21247 21256-21262 21272-21283 21295-21296 21554-21556 21587-21655 21899-21904 21936-21938 21951-21954 21958-21966 22007-22015 22040-22041 22047-22049 22070-22073 22152-22156 22165-22168 22171-22175 22218-22224 22243-22245 22253-22255 22292-22299 22312-22313 22343-22346 22372-22373 |

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| adult spleen | Clontech | SPLc01 | 188-191 546-547 667-668 697-699 792-795 934-935 981-985 1047 1259-1262 1411-1413 1705-1714 1777 2052-2054 2072 2599-2603 2649-2650 3326-3331 3377 3661- |

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| | | | 19743-19751 19756 19759-19763 19768-19772 19804-19808 19814-19822 19855-19856 19921-19929 19933-19937 19939-19946 19948-19949 19953-19957 19962-19963 19965-19967 19972-19981 20015-20017 20029-20043 20048-20065 20074-20079 20087-20094 20099-20102 20106-20113 20120 20122-20127 20130-20132 20137-20148 20167-20179 20189-20194 20208-20214 20235-20240 20250-20253 20265-20270 20274-20278 20289-20296 20299 20305-20307 20316-20320 20328-20330 20345-20354 20391-20400 20415-20417 20425-20431 20437-20451 20485-20490 20497-20498 20501 20510-20520 20524-20535 20537-20545 20554-20567 20575-20578 20614-20619 20642-20643 20676-20681 20686-20688 20698-20711 20726-20732 20752-20753 20758-20766 20789-20797 20806-20812 20819-20821 20824-20849 20853-20854 20858-20863 20865 20881 20890-20900 20922-20923 20929-20935 20943 20952-20956 20963-20972 20977-20988 20991 20999-21004 21027-21045 21060-21066 21069-21087 21097-21114 21122 21145-21151 21202-21207 21223-21226 21233-21235 21248-21252 21256-21271 21277-21294 21301-21303 21326-21334 21340-21342 21351-21352 21377-21397 21408-21409 21434-21439 21447-21450 21458-21461 21477-21482 21495-21500 21516-21530 21587-21655 21913-21921 21929-21935 21967-21968 21973-21974 21978 21989-21993 21995-22018 22020-22029 22042-22044 22050-22059 22074-22076 22092-22094 22097 22115-22116 22141-22143 22152-22156 22160 22187-22192 22195-22198 22204-22226 22246-22255 22271-22289 22292-22299 22314-22318 22323-22328 22333-22335 22343-22348 22358-22359 22365-22371 22383-22388 22399-22408 22434-22435 22440-22448 22495 22534-22539 22553-22558 22560 22571-22581 22599-22602 22607-22609 22622-22628 22634-22643 22653-22654 22661-22662 22700-22701 22707 22741-22742 22760-22762 22768-22770 22805-22816 22823-22829 22833-22834 22846-22849 22854-22858 22870-22874 22927-22938 22948-22950 22955-22957 22969-22971 22991-22994 23047-23050 23059-23060 23066-23067 23070-23071 23074-23076 23085-23086 23098-23101 23117-23119 23123-23124 23218-23219 23242-23244 23249 23251 23261-23262 23358-23360 23382-23385 23392-23395 23439-23447 23470-23472 23489-23493 23510-23511 23514-23518 23524-23525 23532-23539 23543-23544 23682-23686 23704 23718-23719 23761 23771-23780 23793-23797 23806-23809 23827-23833 23843-23844 23856-23860 23871-23877 23890-23892 23906-23910 23941-23956 23999-24001 24005-24011 24014-24018 24026-24028 24056 24126-24129 24772-24774 25306 25316-25318 25323-25325 25334-25335 25340-25341 25370-25375 25534-25536 25966-25968 26024-26028 26195 26209-26213 26235-26236 26248-26252 26294-26302 26307-26309 26316-26321 26327 26337 26341-26344 26349 26359-26365 26373-26374 26383-26385 26401-26402 26405 26411 26424 |

| Tissue origin | RNA Source | Library Name | SEQ ID NOS: |
|---|---|---|---|
| | | | 26440-26445 26453 26596-26602 26615-26616 26732 26737 26796-26798 26843-26845 26850-26852 26858-26862 26876-26879 26891-26892 26980-26984 26988 27048-27049 27052 27074-27077 27091 27105 27122-27126 27147-27149 27209-27213 27229-27232 27269-27270 27275-27276 27414-27431 27439-27443 27468 27486-27492 27496 27498-27500 27510-27511 27589-27592 27602-27606 27622 27636-27639 27649-27654 27658 27662-27672 27698-27699 27726-27739 27744-27748 27767-27768 27816-27818 27840-27844 27848-27852 27861-27864 27890-27892 27896-27927 27989-27990 28019-28038 28097-28100 28142-28147 28161 28192-28198 28204-28209 28290-28292 28311-28313 28315-28316 28361-28362 28365-28370 28420 28424 28435-28439 28464 28468-28484 29117-29123 29278-29283 29309-29314 29332-29337 29364-29366 29378-29379 29940-29953 30135-30142 30150-30156 30195-30199 30224-30232 30240-30242 30361-30368 |
| trachea | Clontech | TRC001 | 177-178 180-181 255-256 395 518-519 546-547 847-848 919-922 934-935 1312-1313 1431 1561 1667-1668 1875-1877 1879-1880 1884-1892 2015-2018 2370-2373 2516 2926 2951-2955 3001 3165-3169 3322-3323 3441-3442 3525 3622-3623 3676 3840-3852 3949-3953 4067-4072 4151-4155 4158-4159 4386-4387 4515-4516 5056 5253-5266 5337-5341 5530-5531 5846 6002-6004 6113 6226-6234 7618-7621 7745-7746 7794 7806-7808 7988 8041-8042 8342 8449-8452 8510-8511 8543-8550 8576-8582 8919-8933 9049-9062 9264-9265 9531 9729-9731 9739-9742 9786-9790 9828 9848-9850 10306 10449-10450 10551-10554 10869 10980-10985 11123-11124 11252-11273 11731-11734 11906-11907 12360 12374-12377 12432-12433 12760-12762 12872-12881 13609-13612 13632-13633 14004 14048-14053 14058-14059 14105-14106 14170-14172 14207-14208 14546-14549 14604 15290-15291 15491-15495 15588-15589 16434-16437 16636-16637 16666-16667 16727-16733 17073 17455-17456 17958-17962 18527 18633-18637 18673-18677 18796 18857-18880 18882-18888 18894-18896 18975 19057-19058 19074-19080 19084-19085 19138-19140 19362 19370 19401-19402 19422-19431 19494-19496 19749-19751 19764-19767 19953-19957 19962-19963 19972-19981 20257-20262 20265-20270 20289-20296 20441-20451 20472-20474 20548-20553 20631-20634 20698-20707 20727-20732 20792-20797 20813-20815 20929-20932 20952-20954 20973-20976 21060-21066 21097-21100 21137-21140 21171-21174 21297-21298 21403-21404 21410-21416 21447-21450 21973-21974 21978 22135-22138 22171-22175 22358-22359 22383-22388 22455-22465 22551-22552 22634-22636 22760-22762 22828-22829 22870-22874 22955-22957 22969-22970 22972 23071 23222 23261-23262 23382-23385 23726-23730 23762-23763 23827-23833 23871-23875 23890-23892 24126-24129 24772-24774 25306 25347-25354 26270-26272 26341-26344 26469-26470 26665-26666 26876-26879 27052 27129-27132 27557-27559 |

| Tissue origin | RNA Source | Library Name | SEQ ID NOS: |
|---|---|---|---|
| | | | 27600-27601 27814-27815 27819-27820 28142-28145 28233 29367 30141-30142 |
| uterus | Clontech | UTR001 | 124-125 188-191 227-230 251-252 301 329-331 738-740 919-922 1028 1047 1453-1454 1562-1564 1705-1714 1893-1901 1912-1913 2366 2374-2377 2926 2988-2989 3001 3205-3207 4557-4560 4712-4713 4971-4974 5903-5906 5919-5921 6114-6136 6235-6237 6403-6404 6533-6535 6625-6626 6932-6938 7678-7680 7728 7771 7798-7801 7921-7923 7946 8010-8012 8084-8085 8090-8093 8137-8141 8313-8314 8368-8370 8415 8420-8421 8689-8690 9072-9074 9264-9265 9517-9521 9535-9537 9577-9578 9828-9832 9848-9850 9929-9935 9953 10033-10037 10268-10272 10508-10512 10537-10538 10980-10985 11071-11075 11135 11505-11506 11546 11609 11731-11734 11803-11804 12023-12026 12046-12049 12190-12191 12378-12397 12432-12433 12894-12897 13107-13117 13592-13597 13888-13895 13954-13956 14058-14059 14261-14263 14445-14447 14604 14650-14651 14988-14992 15182-15183 15187-15189 15290-15291 15390 15576-15577 15699-15700 15855-15857 16145-16146 16174-16176 16600 16643-16648 16716-16723 16851-16853 17330-17332 17454 17958-17962 18015-18016 18527 18655-18658 18673-18677 18761 18789 18825-18834 18894-18896 18899-18903 18936-18939 19036-19039 19074-19083 19362 19370 19375-19379 19387-19389 19442-19444 19560-19562 19609-19615 19693 19727-19732 19764-19767 19816-19818 19926-19929 19933-19937 19950 19981 20029-20043 20120 20122-20127 20146-20148 20151-20154 20289-20296 20298 20328-20330 20366-20368 20401-20405 20427-20431 20469-20471 20491-20494 20554-20557 20602-20606 20629-20630 20649-20652 20689-20692 20753 20758-20767 20801-20805 20858-20862 20864 20938-20942 21005-21008 21072-21075 21213-21215 21281-21294 21377-21397 21911-21912 21955-21957 21978-21982 22019-22025 22050-22055 22090-22091 22187-22192 22218-22224 22251-22252 22261-22264 22358-22359 22362-22364 22373 22405-22408 22571-22581 22622-22624 22644-22651 22663-22664 22887-22891 22955-22957 22969-22970 23047-23050 23094-23097 23141 23425-23427 23439-23447 23543-23544 24029-24033 24130-24144 25085-25090 25340-25341 25374-25375 25416-25417 26221-26223 26270-26272 26285-26290 26327 26607-26609 26676-26677 26755-26756 26853-26854 26860-26862 27173-27174 27294 27348-27353 27493-27496 27602-27606 27636-27639 27649-27654 27729-27739 27861-27864 27896-27927 28105-28121 28133-28137 28311-28313 28424 28426-28428 29339-29340 29378-29379 29962-29963 30150-30156 |

*The 16 tissue-mRNAs and their vendor source, are as follows:  1) Normal adult brain mRNA (Invitrogen), 2) normal adult kidney mRNA (Invitrogen), 3) normal adult liver mRNA (Invitrogen),

4) normal fetal brain mRNA (Invitrogen), 5) normal fetal kidney mRNA (Invitrogen), 6) normal fetal liver mRNA (Invitrogen), 7) normal fetal skin mRNA (Invitrogen), 8) human adrenal gland mRNA (Clontech), 9) human bone marrow mRNA (Clontech), 10) human leukemia lymphablastic mRNA (Clontech), 11) human thymus mRNA (Clontech), 12) human lymph node mRNA (Clontech), 13) human spinal cord mRNA (Clontech), 14) human thyroid mRNA (Clontech), 15) human esophagus mRNA (BioChain), 16) human conceptional umbilical cord mRNA (BioChain).

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Method | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 1 | 30369 | C | 1 | 23 | 76 | |
| 2 | 30370 | B | 2 | 1 | 735 | |
| 3 | 30371 | B | 3 | 1 | 783 | |
| 4 | 30372 | B | 4 | 104 | 266 | |
| 5 | 30373 | B | 5 | 1 | 1113 | |
| 6 | 30374 | C | 6 | 3 | 164 | |
| 7 | 30375 | B | 7 | 112 | 279 | |
| 8 | 30376 | B | 8 | 198 | 405 | |
| 9 | 30377 | B | 9 | 1 | 687 | |
| 10 | 30378 | C | 10 | 346 | 598 | |
| 11 | 30379 | B | 11 | 1 | 960 | |
| 12 | 30380 | B | 12 | 44 | 350 | |
| 13 | 30381 | B | 13 | 264 | 465 | |
| 14 | 30382 | B | 14 | 483 | 1556 | |
| 15 | 30383 | B | 15 | 140 | 838 | |
| 16 | 30384 | B | 16 | 1 | 372 | |
| 17 | 30385 | B | 17 | 1 | 1404 | |
| 18 | 30386 | B | 18 | 25 | 2013 | |
| 19 | 30387 | C | 19 | 1 | 381 | |
| 20 | 30388 | C | 20 | 605 | 755 | |
| 21 | 30389 | B | 21 | 1 | 912 | |
| 22 | 30390 | C | 22 | 124 | 315 | |
| 23 | 30391 | C | 23 | 44 | 310 | |
| 24 | 30392 | B | 24 | 1 | 330 | |
| 25 | 30393 | B | 25 | 1 | 411 | |
| 26 | 30394 | B | 26 | 147 | 257 | |
| 27 | 30395 | B | 27 | 1 | 597 | |
| 28 | 30396 | B | 28 | 201 | 862 | |
| 29 | 30397 | C | 29 | 249 | 515 | |
| 30 | 30398 | B | 30 | 41 | 816 | |
| 31 | 30399 | C | 31 | 26 | 142 | |
| 32 | 30400 | B | 32 | 259 | 2328 | |
| 33 | 30401 | B | 33 | 1 | 759 | |
| 34 | 30402 | B | 34 | 964 | 2121 | |
| 35 | 30403 | C | 35 | 298 | 449 | |
| 36 | 30404 | C | 36 | 115 | 396 | |
| 37 | 30405 | C | 37 | 148 | 318 | |
| 38 | 30406 | C | 38 | 383 | 483 | |
| 39 | 30407 | B | 39 | 1 | 1125 | |
| 40 | 30408 | B | 40 | 1 | 831 | |
| 41 | 30409 | C | 41 | 363 | 602 | |
| 42 | 30410 | B | 42 | 1 | 324 | |
| 43 | 30411 | B | 43 | 64 | 199 | |
| 44 | 30412 | B | 44 | 1 | 1007 | |
| 45 | 30413 | C | 45 | 380 | 583 | |
| 46 | 30414 | B | 46 | 1 | 432 | |
| 47 | 30415 | C | 47 | 1 | 249 | |
| 48 | 30416 | B | 48 | 1 | 798 | |
| 49 | 30417 | B | 49 | 14 | 1070 | |
| 50 | 30418 | C | 50 | 1 | 225 | |
| 51 | 30419 | B | 51 | 1 | 2673 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Method | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 52 | 30420 | B | 52 | 1 | 258 | |
| 53 | 30421 | B | 54 | 1 | 624 | |
| 54 | 30422 | C | 55 | 166 | 333 | |
| 55 | 30423 | B | 56 | 298 | 380 | |
| 56 | 30424 | C | 57 | 139 | 379 | |
| 57 | 30425 | B | 58 | 1 | 157 | |
| 58 | 30426 | B | 59 | 1 | 447 | |
| 59 | 30427 | B | 60 | 1 | 579 | |
| 60 | 30428 | B | 61 | 1 | 1059 | |
| 61 | 30429 | B | 62 | 1 | 816 | |
| 62 | 30430 | B | 63 | 1 | 558 | |
| 63 | 30431 | B | 64 | 1 | 540 | |
| 64 | 30432 | B | 65 | 1 | 555 | |
| 65 | 30433 | B | 66 | 1 | 648 | |
| 66 | 30434 | B | 67 | 1 | 798 | |
| 67 | 30435 | B | 68 | 1 | 1455 | |
| 68 | 30436 | B | 69 | 1 | 1278 | |
| 69 | 30437 | B | 70 | 88 | 3012 | |
| 70 | 30438 | B | 71 | 1 | 1092 | |
| 71 | 30439 | B | 72 | 575 | 1033 | |
| 72 | 30440 | B | 73 | 644 | 926 | |
| 73 | 30441 | B | 74 | 1 | 1239 | |
| 74 | 30442 | B | 75 | 1 | 1074 | |
| 75 | 30443 | B | 76 | 81 | 467 | |
| 76 | 30444 | C | 77 | 44 | 286 | |
| 77 | 30445 | B | 78 | 1 | 297 | |
| 78 | 30446 | B | 79 | 1 | 978 | |
| 79 | 30447 | B | 80 | 72 | 715 | |
| 80 | 30448 | B | 81 | 1 | 1296 | |
| 81 | 30449 | B | 82 | 63 | 162 | |
| 82 | 30450 | C | 83 | 22 | 420 | |
| 83 | 30451 | C | 84 | 201 | 733 | |
| 84 | 30452 | C | 85 | 417 | 575 | |
| 85 | 30453 | B | 86 | 1 | 267 | |
| 86 | 30454 | B | 87 | 112 | 738 | |
| 87 | 30455 | C | 88 | 260 | 379 | |
| 88 | 30456 | B | 89 | 77 | 399 | |
| 89 | 30457 | B | 90 | 158 | 420 | |
| 90 | 30458 | B | 91 | 1 | 1437 | |
| 91 | 30459 | C | 92 | 22 | 321 | |
| 92 | 30460 | B | 93 | 1 | 843 | |
| 93 | 30461 | B | 94 | 142 | 2798 | |
| 94 | 30462 | B | 95 | 887 | 8434 | |
| 95 | 30463 | B | 96 | 1 | 1014 | |
| 96 | 30464 | B | 97 | 1 | 1197 | |
| 97 | 30465 | B | 98 | 16 | 555 | |
| 98 | 30466 | B | 99 | 1 | 423 | |
| 99 | 30467 | B | 100 | 1 | 651 | |
| 100 | 30468 | B | 101 | 233 | 556 | |
| 101 | 30469 | B | 102 | 192 | 883 | |
| 102 | 30470 | C | 103 | 65 | 274 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 103 | 30471 | C | 104 | 328 | 546 | |
| 104 | 30472 | B | 105 | 80 | 3900 | |
| 105 | 30473 | B | 106 | 1 | 951 | |
| 106 | 30474 | C | 107 | 1 | 279 | |
| 107 | 30475 | C | 108 | 246 | 368 | |
| 108 | 30476 | B | 109 | 1 | 819 | |
| 109 | 30477 | B | 110 | 1 | 634 | |
| 110 | 30478 | B | 111 | 1 | 379 | |
| 111 | 30479 | B | 112 | 80 | 2747 | |
| 112 | 30480 | C | 113 | 139 | 414 | |
| 113 | 30481 | C | 114 | 1 | 330 | |
| 114 | 30482 | B | 115 | 53 | 618 | |
| 115 | 30483 | B | 116 | 1 | 426 | |
| 116 | 30484 | C | 117 | 135 | 296 | |
| 117 | 30485 | C | 118 | 239 | 432 | |
| 118 | 30486 | C | 119 | 381 | 776 | |
| 119 | 30487 | B | 120 | 1 | 381 | |
| 120 | 30488 | C | 121 | 42 | 175 | |
| 121 | 30489 | C | 122 | 1 | 399 | |
| 122 | 30490 | B | 123 | 1 | 792 | |
| 123 | 30491 | B | 124 | 1 | 894 | |
| 124 | 30492 | B | 125 | 1 | 3498 | |
| 125 | 30493 | B | 126 | 8 | 874 | |
| 126 | 30494 | B | 127 | 1 | 2160 | |
| 127 | 30495 | B | 128 | 1 | 1776 | |
| 128 | 30496 | B | 129 | 1 | 567 | |
| 129 | 30497 | B | 130 | 195 | 728 | |
| 130 | 30498 | B | 131 | 1 | 615 | |
| 131 | 30499 | B | 132 | 1 | 420 | |
| 132 | 30500 | B | 133 | 661 | 2711 | |
| 133 | 30501 | B | 134 | 1 | 621 | |
| 134 | 30502 | C | 136 | 1 | 465 | |
| 135 | 30503 | C | 137 | 113 | 502 | |
| 136 | 30504 | C | 139 | 78 | 269 | |
| 137 | 30505 | C | 140 | 98 | 472 | |
| 138 | 30506 | B | 141 | 403 | 533 | |
| 139 | 30507 | C | 142 | 64 | 315 | |
| 140 | 30508 | B | 143 | 1 | 591 | |
| 141 | 30509 | C | 144 | 528 | 1151 | |
| 142 | 30510 | C | 145 | 1 | 414 | |
| 143 | 30511 | B | 146 | 1 | 936 | |
| 144 | 30512 | C | 147 | 91 | 195 | |
| 145 | 30513 | C | 148 | 562 | 705 | |
| 146 | 30514 | C | 149 | 122 | 313 | |
| 147 | 30515 | B | 150 | 566 | 1535 | |
| 148 | 30516 | C | 151 | 75 | 248 | |
| 149 | 30517 | C | 152 | 1 | 624 | |
| 150 | 30518 | C | 153 | 551 | 655 | |
| 151 | 30519 | C | 154 | 315 | 497 | |
| 152 | 30520 | C | 155 | 262 | 554 | |
| 153 | 30521 | C | 156 | 1 | 282 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 154 | 30522 | B | 157 | 1 | 508 | |
| 155 | 30523 | C | 158 | 243 | 545 | |
| 156 | 30524 | B | 159 | 8 | 395 | |
| 157 | 30525 | C | 160 | 33 | 194 | |
| 158 | 30526 | B | 161 | 50 | 355 | |
| 159 | 30527 | B | 162 | 128 | 1230 | |
| 160 | 30528 | B | 163 | 243 | 710 | |
| 161 | 30529 | B | 164 | 121 | 742 | |
| 162 | 30530 | B | 165 | 152 | 227 | |
| 163 | 30531 | C | 166 | 156 | 503 | |
| 164 | 30532 | B | 167 | 67 | 1280 | |
| 165 | 30533 | B | 168 | 1 | 444 | |
| 166 | 30534 | B | 169 | 161 | 206 | |
| 167 | 30535 | B | 170 | 189 | 1207 | |
| 168 | 30536 | B | 171 | 1 | 613 | |
| 169 | 30537 | B | 172 | 1 | 70 | |
| 170 | 30538 | C | 173 | 611 | 751 | |
| 171 | 30539 | B | 174 | 398 | 2472 | |
| 172 | 30540 | B | 175 | 87 | 646 | |
| 173 | 30541 | B | 176 | 1 | 1455 | |
| 174 | 30542 | C | 177 | 1 | 339 | |
| 175 | 30543 | B | 178 | 1 | 1458 | |
| 176 | 30544 | B | 179 | 278 | 766 | |
| 177 | 30545 | B | 181 | 85 | 749 | |
| 178 | 30546 | B | 182 | 50 | 498 | |
| 179 | 30547 | C | 183 | 1 | 522 | |
| 180 | 30548 | B | 184 | 90 | 482 | |
| 181 | 30549 | B | 185 | 86 | 442 | |
| 182 | 30550 | C | 187 | 129 | 308 | |
| 183 | 30551 | C | 188 | 1 | 414 | |
| 184 | 30552 | B | 190 | 1 | 378 | |
| 185 | 30553 | C | 192 | 252 | 308 | |
| 186 | 30554 | B | 193 | 1 | 576 | |
| 187 | 30555 | C | 194 | 1093 | 1311 | |
| 188 | 30556 | B | 195 | 45 | 324 | |
| 189 | 30557 | B | 196 | 1 | 249 | |
| 190 | 30558 | C | 197 | 309 | 443 | |
| 191 | 30559 | C | 198 | 615 | 866 | |
| 192 | 30560 | B | 199 | 86 | 1332 | |
| 193 | 30561 | B | 200 | 49 | 334 | |
| 194 | 30562 | B | 201 | 64 | 638 | |
| 195 | 30563 | C | 202 | 195 | 338 | |
| 196 | 30564 | C | 203 | 1 | 357 | |
| 197 | 30565 | B | 204 | 1 | 693 | |
| 198 | 30566 | C | 205 | 121 | 291 | |
| 199 | 30567 | C | 206 | 156 | 380 | |
| 200 | 30568 | C | 207 | 1211 | 1456 | |
| 201 | 30569 | B | 208 | 62 | 328 | |
| 202 | 30570 | C | 209 | 105 | 179 | |
| 203 | 30571 | B | 210 | 229 | 1483 | |
| 204 | 30572 | B | 211 | 1 | 749 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 205 | 30573 | B | 212 | 1 | 190 | |
| 206 | 30574 | C | 213 | 121 | 367 | |
| 207 | 30575 | B | 214 | 121 | 456 | |
| 208 | 30576 | B | 215 | 1 | 2631 | |
| 209 | 30577 | B | 216 | 63 | 419 | |
| 210 | 30578 | B | 217 | 114 | 485 | |
| 211 | 30579 | B | 218 | 628 | 1447 | |
| 212 | 30580 | C | 219 | 252 | 377 | |
| 213 | 30581 | B | 220 | 1 | 847 | |
| 214 | 30582 | B | 221 | 68 | 343 | |
| 215 | 30583 | B | 222 | 138 | 911 | |
| 216 | 30584 | B | 223 | 44 | 882 | |
| 217 | 30585 | B | 224 | 1 | 429 | |
| 218 | 30586 | B | 225 | 87 | 312 | |
| 219 | 30587 | C | 226 | 44 | 343 | |
| 220 | 30588 | C | 227 | 41 | 286 | |
| 221 | 30589 | C | 228 | 1145 | 1372 | |
| 222 | 30590 | B | 229 | 1 | 720 | |
| 223 | 30591 | C | 230 | 1 | 430 | |
| 224 | 30592 | C | 231 | 58 | 297 | |
| 225 | 30593 | B | 232 | 613 | 683 | |
| 226 | 30594 | B | 233 | 613 | 683 | |
| 227 | 30595 | C | 234 | 238 | 455 | |
| 228 | 30596 | B | 235 | 319 | 615 | |
| 229 | 30597 | C | 236 | 255 | 494 | |
| 230 | 30598 | B | 237 | 106 | 600 | |
| 231 | 30599 | B | 238 | 1 | 654 | |
| 232 | 30600 | B | 239 | 1 | 654 | |
| 233 | 30601 | B | 240 | 243 | 356 | |
| 234 | 30602 | B | 241 | 1 | 932 | |
| 235 | 30603 | C | 242 | 36 | 215 | |
| 236 | 30604 | B | 243 | 1 | 288 | |
| 237 | 30605 | C | 244 | 25 | 186 | |
| 238 | 30606 | B | 245 | 1 | 574 | |
| 239 | 30607 | B | 246 | 1 | 1257 | |
| 240 | 30608 | B | 247 | 162 | 263 | |
| 241 | 30609 | C | 248 | 79 | 207 | |
| 242 | 30610 | B | 249 | 194 | 276 | |
| 243 | 30611 | B | 250 | 1 | 1671 | |
| 244 | 30612 | C | 251 | 118 | 311 | |
| 245 | 30613 | B | 252 | 88 | 1485 | |
| 246 | 30614 | B | 253 | 339 | 443 | |
| 247 | 30615 | B | 254 | 667 | 1165 | |
| 248 | 30616 | B | 255 | 1 | 981 | |
| 249 | 30617 | B | 256 | 450 | 3131 | |
| 250 | 30618 | B | 257 | 900 | 1199 | |
| 251 | 30619 | C | 258 | 5 | 271 | |
| 252 | 30620 | B | 259 | 65 | 689 | |
| 253 | 30621 | C | 260 | 1 | 321 | |
| 254 | 30622 | B | 261 | 1 | 137 | |
| 255 | 30623 | B | 262 | 34 | 282 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 256 | 30624 | B | 263 | 46 | 856 | |
| 257 | 30625 | C | 264 | 157 | 468 | |
| 258 | 30626 | B | 265 | 148 | 403 | |
| 259 | 30627 | C | 266 | 248 | 481 | |
| 260 | 30628 | B | 267 | 171 | 393 | |
| 261 | 30629 | B | 268 | 1 | 1078 | |
| 262 | 30630 | B | 269 | 1 | 550 | |
| 263 | 30631 | B | 270 | 1 | 1455 | |
| 264 | 30632 | B | 271 | 171 | 602 | |
| 265 | 30633 | B | 272 | 1 | 1056 | |
| 266 | 30634 | B | 273 | 1 | 1101 | |
| 267 | 30635 | B | 274 | 1 | 2335 | |
| 268 | 30636 | B | 275 | 303 | 419 | |
| 269 | 30637 | B | 276 | 1 | 615 | |
| 270 | 30638 | B | 277 | 1 | 543 | |
| 271 | 30639 | B | 278 | 1 | 1602 | |
| 272 | 30640 | C | 279 | 585 | 1001 | |
| 273 | 30641 | C | 280 | 260 | 379 | |
| 274 | 30642 | B | 281 | 1 | 1437 | |
| 275 | 30643 | C | 282 | 22 | 321 | |
| 276 | 30644 | B | 283 | 1 | 843 | |
| 277 | 30645 | B | 284 | 142 | 2796 | |
| 278 | 30646 | B | 285 | 458 | 7217 | |
| 279 | 30647 | B | 286 | 84 | 186 | |
| 280 | 30648 | C | 287 | 67 | 229 | |
| 281 | 30649 | C | 288 | 15 | 245 | |
| 282 | 30650 | C | 289 | 125 | 232 | |
| 283 | 30651 | B | 290 | 1 | 594 | |
| 284 | 30652 | B | 291 | 376 | 670 | |
| 285 | 30653 | C | 292 | 82 | 405 | |
| 286 | 30654 | B | 293 | 35 | 651 | |
| 287 | 30655 | B | 294 | 56 | 487 | |
| 288 | 30656 | C | 295 | 313 | 498 | |
| 289 | 30657 | C | 296 | 118 | 261 | |
| 290 | 30658 | B | 297 | 198 | 1868 | |
| 291 | 30659 | B | 298 | 1 | 1665 | |
| 292 | 30660 | C | 299 | 73 | 108 | |
| 293 | 30661 | B | 300 | 1 | 408 | |
| 294 | 30662 | B | 301 | 1 | 444 | |
| 295 | 30663 | B | 302 | 8 | 311 | |
| 296 | 30664 | C | 303 | 144 | 350 | |
| 297 | 30665 | B | 304 | 1 | 669 | |
| 298 | 30666 | C | 305 | 416 | 820 | |
| 299 | 30667 | B | 306 | 253 | 837 | |
| 300 | 30668 | B | 307 | 44 | 475 | |
| 301 | 30669 | B | 308 | 185 | 885 | |
| 302 | 30670 | C | 309 | 206 | 337 | |
| 303 | 30671 | B | 310 | 1 | 393 | |
| 304 | 30672 | B | 311 | 1 | 1259 | |
| 305 | 30673 | B | 312 | 24 | 434 | |
| 306 | 30674 | B | 313 | 44 | 2687 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 307 | 30675 | B | 314 | 1 | 154 | |
| 308 | 30676 | B | 315 | 288 | 770 | |
| 309 | 30677 | B | 316 | 85 | 683 | |
| 310 | 30678 | B | 317 | 1 | 873 | |
| 311 | 30679 | B | 318 | 1 | 1737 . | |
| 312 | 30680 | C | 319 | 1 | 690 | |
| 313 | 30681 | B | 320 | 58 | 1487 | |
| 314 | 30682 | B | 321 | 1 | 816 | |
| 315 | 30683 | B | 322 | 25 | 772 | |
| 316 | 30684 | B | 323 | 42 | 271 | |
| 317 | 30685 | C | 324 | 16 | 159 | |
| 318 | 30686 | C | 325 | 74 | 280 | |
| 319 | 30687 | C | 326 | 221 | 545 | |
| 320 | 30688 | B | 327 | 192 | 364 | |
| 321 | 30689 | C | 328 | 390 | 638 | |
| 322 | 30690 | B | 329 | 151 | 4215 | |
| 323 | 30691 | B | 330 | 1 | 2076 | |
| 324 | 30692 | B | 331 | 1 | 465 | |
| 325 | 30693 | B | 332 | 40 | 1350 | |
| 326 | 30694 | B | 333 | 1 | 489 | |
| 327 | 30695 | B | 334 | 285 | 744 | |
| 328 | 30696 | C | 335 | 96 | 347 | |
| 329 | 30697 | C | 336 | 213 | 326 | |
| 330 | 30698 | B | 337 | 776 | 4384 | |
| 331 | 30699 | B | 338 | 201 | 317 | |
| 332 | 30700 | B | 339 | 1 | 2713 | |
| 333 | 30701 | B | 340 | 1 | 894 | |
| 334 | 30702 | B | 341 | 1 | 3842 | |
| 335 | 30703 | C | 342 | 745 | 1131 | |
| 336 | 30704 | B | 343 | 82 | 411 | |
| 337 | 30705 | B | 344 | 126 | 2123 | |
| 338 | 30706 | B | 345 | 57 | 1641 | |
| 339 | 30707 | C | 346 | 211 | 654 | |
| 340 | 30708 | B | 347 | 44 | 266 | |
| 341 | 30709 | B | 348 | 1 | 927 | |
| 342 | 30710 | C | 349 | 20 | 124 | |
| 343 | 30711 | C | 350 | 9 | 455 | |
| 344 | 30712 | C | 351 | 188 | 304 | |
| 345 | 30713 | C | 352 | 1 | 333 | |
| 346 | 30714 | C | 353 | 140 | 298 | |
| 347 | 30715 | B | 354 | 73 | 2171 | |
| 348 | 30716 | B | 355 | 1 | 1374 | |
| 349 | 30717 | B | 356 | 150 | 398 | |
| 350 | 30718 | B | 357 | 1 | 585 | |
| 351 | 30719 | B | 358 | 1 | 1716 | |
| 352 | 30720 | B | 359 | 81 | 1912 | |
| 353 | 30721 | B | 360 | 249 | 770 | |
| 354 | 30722 | B | 361 | 474 | 2875 | |
| 355 | 30723 | C | 362 | 1 | 483 | |
| 356 | 30724 | C | 363 | 1 | 251 | |
| 357 | 30725 | C | 364 | 28 | 407 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleutide insertion) |
|---|---|---|---|---|---|---|
| 358 | 30726 | C | 365 | 88 | 204 | |
| 359 | 30727 | B | 366 | 474 | 684 | |
| 360 | 30728 | C | 367 | 41 | 394 | |
| 361 | 30729 | B | 368 | 253 | 1044 | |
| 362 | 30730 | B | 369 | 468 | 1111 | |
| 363 | 30731 | B | 370 | 1 | 558 | |
| 364 | 30732 | B | 371 | 21 | 345 | |
| 365 | 30733 | B | 372 | 1 | 744 | |
| 366 | 30734 | B | 373 | 1 | 795 | |
| 367 | 30735 | B | 374 | 1 | 685 | |
| 368 | 30736 | B | 375 | 94 | 414 | |
| 369 | 30737 | C | 376 | 86 | 268 | |
| 370 | 30738 | B | 377 | 1 | 1003 | |
| 371 | 30739 | B | 378 | 41 | 1385 | |
| 372 | 30740 | B | 379 | 1 | 510 | |
| 373 | 30741 | B | 380 | 40 | 746 | |
| 374 | 30742 | B | 381 | 100 | 1991 | |
| 375 | 30743 | B | 382 | 1 | 267 | |
| 376 | 30744 | C | 383 | 168 | 278 | |
| 377 | 30745 | C | 384 | 173 | 208 | |
| 378 | 30746 | B | 385 | 141 | 4538 | |
| 379 | 30747 | B | 386 | 1 | 4086 | |
| 380 | 30748 | C | 387 | 398 | 474 | |
| 381 | 30749 | B | 388 | 1 | 762 | |
| 382 | 30750 | B | 389 | 1 | 1584 | |
| 383 | 30751 | B | 390 | 1 | 2703 | |
| 384 | 30752 | B | 391 | 1 | 489 | |
| 385 | 30753 | B | 392 | 527 | 780 | |
| 386 | 30754 | B | 393 | 1 | 4050 | |
| 387 | 30755 | B | 394 | 859 | 2958 | |
| 388 | 30756 | B | 395 | 639 | 2307 | |
| 389 | 30757 | B | 396 | 1 | 642 | |
| 390 | 30758 | B | 397 | 1 | 3639 | |
| 391 | 30759 | B | 398 | 219 | 540 | |
| 392 | 30760 | B | 399 | 1 | 3225 | |
| 393 | 30761 | B | 400 | 1 | 7552 | |
| 394 | 30762 | C | 401 | 626 | 1201 | |
| 395 | 30763 | C | 402 | 627 | 827 | |
| 396 | 30764 | C | 403 | 1 | 243 | |
| 397 | 30765 | B | 404 | 335 | 538 | |
| 398 | 30766 | B | 405 | 41 | 409 | |
| 399 | 30767 | B | 406 | 160 | 540 | |
| 400 | 30768 | B | 407 | 1 | 597 | |
| 401 | 30769 | B | 408 | 1 | 1605 | |
| 402 | 30770 | B | 409 | 1 | 351 | |
| 403 | 30771 | B | 410 | 65 | 601 | |
| 404 | 30772 | B | 411 | 1 | 870 | |
| 405 | 30773 | B | 412 | 91 | 2867 | |
| 406 | 30774 | B | 413 | 33 | 410 | |
| 407 | 30775 | B | 414 | 298 | 343 | |
| 408 | 30776 | B | 415 | 70 | 310 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 409 | 30777 | B | 416 | 64 | 1929 | |
| 410 | 30778 | B | 417 | 1 | 298 | |
| 411 | 30779 | B | 418 | 37 | 2612 | |
| 412 | 30780 | B | 419 | 1 | 510 | |
| 413 | 30781 | B | 420 | 44 | 1111 | |
| 414 | 30782 | B | 421 | 26 | 175 | |
| 415 | 30783 | C | 422 | 7 | 57 | |
| 416 | 30784 | C | 423 | 27 | 230 | |
| 417 | 30785 | C | 424 | 7 | 144 | |
| 418 | 30786 | B | 425 | 1 | 1746 | |
| 419 | 30787 | C | 426 | 318 | 486 | |
| 420 | 30788 | C | 427 | 896 | 1115 | |
| 421 | 30789 | C | 428 | 106 | 309 | |
| 422 | 30790 | C | 429 | 52 | 402 | |
| 423 | 30791 | B | 430 | 1 | 309 | |
| 424 | 30792 | B | 431 · | 167 | 492 | |
| 425 | 30793 | C | 432 | 144 | 296 | |
| 426 | 30794 | B | 433 | 1 | 786 | |
| 427 | 30795 | B | 434 | 336 | 1303 | |
| 428 | 30796 | B | 435 | 333 | 419 | |
| 429 | 30797 | B | 436 | 1 | 489 | |
| 430 | 30798 | C | 437 | 1 | 199 | |
| 431 | 30799 | C | 438 | 110 | 239 | |
| 432 | 30800 | C | 439 | 175 | 303 | |
| 433 | 30801 | C | 440 | 35 | 181 | |
| 434 | 30802 | B | 441 | 1 | 1896 | |
| 435 | 30803 | C | 442 | 1 | 331 | |
| 436 | 30804 | C | 443 | 71 | 344 | |
| 437 | 30805 | C | 444 | 25 | 135 | |
| 438 | 30806 | C | 445 | 406 | 595 | |
| 439 | 30807 | C | 446 | 148 | 228 | |
| 440 | 30808 | C | 447 | 80 | 106 | |
| 441 | 30809 | C | 448 | 7 | 375 | |
| 442 | 30810 | C | 449 | 300 | 437 | |
| 443 | 30811 | C | 450 | 1 | 357 | |
| 444 | 30812 | B | 451 | 1 | 729 | |
| 445 | 30813 | B | 452 | 58 | 1287 | |
| 446 | 30814 | C | 453 | 1 | 410 | |
| 447 | 30815 | C | 454 | 1 | 411 | |
| 448 | 30816 | C | 455 | 1 | 420 | |
| 449 | 30817 | B | 456 | 1 | 555 | |
| 450 | 30818 | B | 457 | 376 | 1035 | |
| 451 | 30819 | B | 458 | 678 | 807 | |
| 452 | 30820 | B | 459 | 88 | 1485 | |
| 453 | 30821 | B | 460 | 300 | 2082 | |
| 454 | 30822 | B | 461 | 1 | 819 | |
| 455 | 30823 | B | 462 | 780 | 998 | |
| 456 | 30824 | B | 463 | 1 | 1871 | |
| 457 | 30825 | B | 464 | 1 | 1703 | |
| 458 | 30826 | B | 465 | 1 | 594 | |
| 459 | 30827 | C | 466 | 120 | 245 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 460 | 30828 | C | 467 | 1 | 387 | |
| 461 | 30829 | B | 468 | 1 | 1678 | |
| 462 | 30830 | B | 469 | 1 | 533 | |
| 463 | 30831 | B | 470 | 347 | 656 | |
| 464 | 30832 | B | 471 | 1 | 1098 | |
| 465 | 30833 | B | 472 | 224 | 1518 | |
| 466 | 30834 | C | 473 | 44 | 244 | |
| 467 | 30835 | B | 474 | 1 | 1251 | |
| 468 | 30836 | B | 475 | 1 | 428 | |
| 469 | 30837 | B | 476 | 1 | 495 | |
| 470 | 30838 | C | 477 | 233 | 373 | |
| 471 | 30839 | B | 478 | 8 | 950 | |
| 472 | 30840 | C | 479 | 1 | 813 | |
| 473 | 30841 | B | 480 | 1 | 1071 | |
| 474 | 30842 | C | 481 | 224 | 418 | |
| 475 | 30843 | B | 482 | 39 | 851 | |
| 476 | 30844 | B | 483 | 1 | 2006 | |
| 477 | 30845 | B | 484 | 1 | 561 | |
| 478 | 30846 | B | 485 | 167 | 227 | |
| 479 | 30847 | B | 486 | 1 | 777 | |
| 480 | 30848 | B | 487 | 1 | 645 | |
| 481 | 30849 | B | 488 | 1 | 1749 | |
| 482 | 30850 | C | 489 | 26 | 847 | |
| 483 | 30851 | C | 490 | 243 | 392 | |
| 484 | 30852 | C | 491 | 303 | 407 | |
| 485 | 30853 | C | 492 | 23 | 300 | |
| 486 | 30854 | B | 493 | 131 | 336 | |
| 487 | 30855 | C | 494 | 64 | 156 | |
| 488 | 30856 | B | 495 | 180 | 712 | |
| 489 | 30857 | B | 496 | 1 | 1104 | |
| 490 | 30858 | B | 497 | 24 | 917 | |
| 491 | 30859 | B | 498 | 65 | 228 | |
| 492 | 30860 | B | 499 | 1 | 2172 | |
| 493 | 30861 | B | 500 | 1 | 1338 | |
| 494 | 30862 | B | 501 | 1 | 795 | |
| 495 | 30863 | C | 502 | 181 | 410 | |
| 496 | 30864 | B | 503 | 69 | 1322 | |
| 497 | 30865 | B | 504 | 531 | 1315 | |
| 498 | 30866 | C | 505 | 24 | 320 | |
| 499 | 30867 | B | 506 | 1 | 791 | |
| 500 | 30868 | B | 507 | 1 | 3256 | |
| 501 | 30869 | C | 508 | 361 | 549 | |
| 502 | 30870 | B | 509 | 729 | 3252 | |
| 503 | 30871 | B | 510 | 424 | 1710 | |
| 504 | 30872 | C | 511 | 247 | 750 | |
| 505 | 30873 | B | 512 | 11 | 124 | |
| 506 | 30874 | B | 514 | 116 | 1079 | |
| 507 | 30875 | B | 515 | 1 | 766 | |
| 508 | 30876 | B | 516 | 185 | 796 | |
| 509 | 30877 | B | 517 | 1 | 456 | |
| 510 | 30878 | B | 518 | 99 | 435 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 511 | 30879 | B | 519 | 1 | 834 | |
| 512 | 30880 | B | 520 | 54 | 246 | |
| 513 | 30881 | B | 521 | 1 | 372 | |
| 514 | 30882 | C | 522 | 78 | 305 | |
| 515 | 30883 | C | 523 | 329 | 484 | |
| 516 | 30884 | B | 524 | 1 | 459 | |
| 517 | 30885 | B | 525 | 630 | 889 | |
| 518 | 30886 | B | 526 | 95 | 343 | |
| 519 | 30887 | B | 527 | 353 | 610 | |
| 520 | 30888 | B | 528 | 113 | 529 | |
| 521 | 30889 | B | 529 | 362 | 1400 | |
| 522 | 30890 | B | 530 | 1 | 441 | |
| 523 | 30891 | C | 531 | 1 | 327 | |
| 524 | 30892 | B | 532 | 1 | 909 | |
| 525 | 30893 | B | 534 | 669 | 1268 | |
| 526 | 30894 | B | 535 | 293 | 826 | |
| 527 | 30895 | C | 536 | 12 | 155 | |
| 528 | 30896 | C | 537 | 1488 | 1706 | |
| 529 | 30897 | C | 538 | 26 | 211 | |
| 530 | 30898 | C | 539 | 30 | 185 | |
| 531 | 30899 | B | 540 | 1 | 789 | |
| 532 | 30900 | B | 541 | 63 | 358 | |
| 533 | 30901 | B | 542 | 1 | 900 | |
| 534 | 30902 | B | 543 | 1 | 728 | |
| 535 | 30903 | B | 544 | 112 | 220 | |
| 536 | 30904 | B | 545 | 49 | 386 | |
| 537 | 30905 | B | 546 | 1 | 585 | |
| 538 | 30906 | B | 547 | 328 | 531 | |
| 539 | 30907 | B | 548 | 10 | 987 | |
| 540 | 30908 | B | 549 | 49 | 248 | |
| 541 | 30909 | B | 550 | 131 | 368 | |
| 542 | 30910 | B | 551 | 80 | 1098 | |
| 543 | 30911 | B | 552 | 1 | 1364 | |
| 544 | 30912 | B | 553 | 1 | 1294 | |
| 545 | 30913 | B | 554 | 1 | 1995 | |
| 546 | 30914 | B | 555 | 1 | 279 | |
| 547 | 30915 | B | 556 | 175 | 715 | |
| 548 | 30916 | B | 557 | 1 | 636 | |
| 549 | 30917 | B | 558 | 1331 | 1600 | |
| 550 | 30918 | B | 559 | 32 | 406 | |
| 551 | 30919 | B | 560 | 38 | 206 | |
| 552 | 30920 | B | 561 | 1 | 1266 | |
| 553 | 30921 | C | 562 | 359 | 501 | |
| 554 | 30922 | B | 563 | 315 | 465 | |
| 555 | 30923 | B | 564 | 94 | 1683 | |
| 556 | 30924 | B | 565 | 1 | 1570 | |
| 557 | 30925 | B | 566 | 139 | 1734 | |
| 558 | 30926 | B | 567 | 1 | 810 | |
| 559 | 30927 | B | 568 | 658 | 1548 | |
| 560 | 30928 | B | 569 | 9 | 395 | |
| 561 | 30929 | B | 570 | 1 | 567 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 562 | 30930 | B | 571 | 1 | 567 | |
| 563 | 30931 | B | 572 | 1 | 789 | |
| 564 | 30932 | B | 573 | 49 | 3187 | |
| 565 | 30933 | B | 574 | 1 | 1824 | |
| 566 | 30934 | B | 575 | 49 | 1413 | |
| 567 | 30935 | B | 576 | 1 | 1572 | |
| 568 | 30936 | C | 577 | 372 | 468 | |
| 569 | 30937 | C | 578 | 58 | 225 | |
| 570 | 30938 | B | 579 | 79 | 299 | |
| 571 | 30939 | B | 580 | 1 | 645 | |
| 572 | 30940 | C | 581 | 582 | 749 | |
| 573 | 30941 | B | 582 | 170 | 463 | |
| 574 | 30942 | B | 583 | 311 | 520 | |
| 575 | 30943 | B | 584 | 1 | 1074 | |
| 576 | 30944 | B | 585 | 39 | 140 | |
| 577 | 30945 | B | 586 | 60 | 1685 | |
| 578 | 30946 | B | 587 | 106 | 879 | |
| 579 | 30947 | C | 588 | 67 | 362 | |
| 580 | 30948 | B | 589 | 45 | 126 | |
| 581 | 30949 | C | 590 | 1 | 390 | |
| 582 | 30950 | C | 591 | 49 | 240 | |
| 583 | 30951 | B | 592 | 1 | 496 | |
| 584 | 30952 | B | 593 | 94 | 482 | |
| 585 | 30953 | C | 594 | 12 | 341 | |
| 586 | 30954 | B | 595 | 1 | 354 | |
| 587 | 30955 | B | 596 | 1 | 711 | |
| 588 | 30956 | B | 597 | 123 | 412 | |
| 589 | 30957 | B | 598 | 1 | 1107 | |
| 590 | 30958 | B | 599 | 1 | 800 | |
| 591 | 30959 | C | 600 | 82 | 408 | |
| 592 | 30960 | B | 601 | 1 | 3174 | |
| 593 | 30961 | B | 602 | 1 | 444 | |
| 594 | 30962 | B | 603 | 1 | 1671 | |
| 595 | 30963 | B | 604 | 1 | 603 | |
| 596 | 30964 | B | 605 | 339 | 443 | |
| 597 | 30965 | C | 606 | 237 | 380 | |
| 598 | 30966 | B | 607 | 1 | 771 | |
| 599 | 30967 | B | 608 | 1 | 1767 | |
| 600 | 30968 | C | 609 | 1 | 801 | |
| 601 | 30969 | B | 610 | 1 | 1062 | |
| 602 | 30970 | B | 611 | 450 | 3131 | |
| 603 | 30971 | C | 612 | 178 | 435 | |
| 604 | 30972 | C | 613 | 164 | 319 | |
| 605 | 30973 | C | 614 | 1 | 385 | |
| 606 | 30974 | C | 615 | 392 | 853 | |
| 607 | 30975 | C | 616 | 24 | 200 | |
| 608 | 30976 | C | 617 | 34 | 327 | |
| 609 | 30977 | B | 618 | 1 | 624 | |
| 610 | 30978 | B | 619 | 179 | 1222 | |
| 611 | 30979 | B | 620 | 1 | 916 | |
| 612 | 30980 | B | 621 | 151 | 339 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Method | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 613 | 30981 | B | 622 | 135 | 218 | |
| 614 | 30982 | B | 623 | 126 | 300 | |
| 615 | 30983 | C | 624 | 258 | 467 | |
| 616 | 30984 | B | 625 | 58 | 1038 | |
| 617 | 30985 | B | 626 | 246 | 4677 | |
| 618 | 30986 | B | 627 | 1 | 583 | |
| 619 | 30987 | C | 628 | 65 | 283 | |
| 620 | 30988 | B | 629 | 162 | 909 | |
| 621 | 30989 | B | 630 | 1 | 1062 | |
| 622 | 30990 | B | 631 | 1 | 909 | |
| 623 | 30991 | C | 632 | 160 | 297 | |
| 624 | 30992 | B | 633 | 352 | 1143 | |
| 625 | 30993 | C | 634 | 301 | 459 | |
| 626 | 30994 | B | 635 | 1 | 906 | |
| 627 | 30995 | B | 636 | 1 | 654 | |
| 628 | 30996 | B | 637 | 1 | 528 | |
| 629 | 30997 | B | 638 | 1 | 1102 | |
| 630 | 30998 | C | 639 | 81 | 299 | |
| 631 | 30999 | B | 640 | 1 | 345 | |
| 632 | 31000 | B | 641 | 39 | 360 | |
| 633 | 31001 | B | 642 | 22 | 293 | |
| 634 | 31002 | C | 643 | 1 | 504 | |
| 635 | 31003 | B | 644 | 107 | 3786 | |
| 636 | 31004 | B | 645 | 1 | 576 | |
| 637 | 31005 | B | 646 | 66 | 152 | |
| 638 | 31006 | B | 647 | 226 | 522 | |
| 639 | 31007 | B | 648 | 1 | 49 | |
| 640 | 31008 | C | 649 | 50 | 172 | |
| 641 | 31009 | C | 650 | 1 | 516 | |
| 642 | 31010 | B | 651 | 1 | 615 | |
| 643 | 31011 | B | 652 | 1 | 495 | |
| 644 | 31012 | B | 653 | 1 | 663 | |
| 645 | 31013 | B | 654 | 1 | 1812 | |
| 646 | 31014 | B | 655 | 1 | 1401 | |
| 647 | 31015 | B | 656 | 102 | 1151 | |
| 648 | 31016 | B | 657 | 1 | 385 | |
| 649 | 31017 | B | 658 | 232 | 987 | |
| 650 | 31018 | B | 659 | 1 | 1221 | |
| 651 | 31019 | B | 660 | 296 | 496 | |
| 652 | 31020 | B | 661 | 57 | 285 | |
| 653 | 31021 | C | 662 | 203 | 271 | |
| 654 | 31022 | B | 663 | 1 | 711 | |
| 655 | 31023 | C | 664 | 351 | 542 | |
| 656 | 31024 | C | 665 | 420 | 695 | |
| 657 | 31025 | B | 666 | 1 | 1860 | |
| 658 | 31026 | B | 667 | 71 | 2167 | |
| 659 | 31027 | B | 668 | 6 | 344 | |
| 660 | 31028 | B | 669 | 217 | 693 | |
| 661 | 31029 | C | 670 | 1 | 417 | |
| 662 | 31030 | B | 671 | 1 | 990 | |
| 663 | 31031 | B | 672 | 109 | 1169 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 664 | 31032 | C | 673 | 40 | 117 | |
| 665 | 31033 | C | 674 | 301 | 560 | |
| 666 | 31034 | B | 675 | 1 | 396 | |
| 667 | 31035 | B | 676 | 483 | 1033 | |
| 668 | 31036 | B | 677 | 673 | 3407 | |
| 669 | 31037 | B | 678 | 4 | 672 | |
| 670 | 31038 | C | 679 | 39 | 116 | |
| 671 | 31039 | B | 680 | 1 | 459 | |
| 672 | 31040 | B | 681 | 19 | 370 | |
| 673 | 31041 | B | 682 | 112 | 704 | |
| 674 | 31042 | C | 683 | 387 | 578 | |
| 675 | 31043 | B | 684 | 175 | 254 | |
| 676 | 31044 | B | 685 | 1 | 501 | |
| 677 | 31045 | B | 686 | 290 | 389 | |
| 678 | 31046 | B | 687 | 1 | 486 | |
| 679 | 31047 | B | 688 | 1 | 651 | |
| 680 | 31048 | B | 689 | 181 | 401 | |
| 681 | 31049 | B | 690 | 117 | 406 | |
| 682 | 31050 | B | 691 | 1 | 169 | |
| 683 | 31051 | B | 692 | 1 | 1539 | |
| 684 | 31052 | B | 693 | 1 | 475 | |
| 685 | 31053 | B | 694 | 1 | 1575 | |
| 686 | 31054 | B | 695 | 1 | 507 | |
| 687 | 31055 | B | 696 | 1 | 498 | |
| 688 | 31056 | C | 697 | 253 | 492 | |
| 689 | 31057 | B | 698 | 1 | 588 | |
| 690 | 31058 | B | 699 | 75 | 291 | |
| 691 | 31059 | B | 700 | 1 | 1355 | |
| 692 | 31060 | B | 701 | 112 | 259 | |
| 693 | 31061 | C | 702 | 492 | 833 | |
| 694 | 31062 | B | 703 | 297 | 483 | |
| 695 | 31063 | B | 704 | 45 | 471 | |
| 696 | 31064 | C | 705 | 175 | 318 | |
| 697 | 31065 | B | 706 | 1 | 1074 | |
| 698 | 31066 | B | 707 | 94 | 1180 | |
| 699 | 31067 | B | 708 | 1 | 3866 | |
| 700 | 31068 | C | 709 | 215 | 424 | |
| 701 | 31069 | B | 710 | 1 | 499 | |
| 702 | 31070 | B | 711 | 210 | 325 | |
| 703 | 31071 | B | 712 | 1 | 786 | |
| 704 | 31072 | B | 713 | 1 | 777 | |
| 705 | 31073 | B | 714 | 174 | 1804 | |
| 706 | 31074 | B | 715 | 17 | 368 | |
| 707 | 31075 | B | 716 | 769 | 1831 | |
| 708 | 31076 | B | 717 | 76 | 301 | |
| 709 | 31077 | B | 718 | 1 | 825 | |
| 710 | 31078 | C | 719 | 1 | 396 | |
| 711 | 31079 | B | 720 | 93 | 2449 | |
| 712 | 31080 | B | 721 | 408 | 687 | |
| 713 | 31081 | B | 722 | 97 | 662 | |
| 714 | 31082 | B | 723 | 169 | 610 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 715 | 31083 | B | 724 | 1 | 2511 | |
| 716 | 31084 | C | 725 | 104 | 410 | |
| 717 | 31085 | C | 726 | 75 | 527 | |
| 718 | 31086 | C | 727 | 7 | 263 | |
| 719 | 31087 | B | 728 | 40 | 1725 | |
| 720 | 31088 | B | 729 | 290 | 1671 | |
| 721 | 31089 | B | 730 | 46 | 465 | |
| 722 | 31090 | C | 731 | 378 | 644 | |
| 723 | 31091 | B | 732 | 48 | 2331 | |
| 724 | 31092 | B | 733 | 1 | 738 | |
| 725 | 31093 | B | 734 | 1 | 1051 | |
| 726 | 31094 | B | 735 | 1 | 840 | |
| 727 | 31095 | C | 736 | 291 | 551 | |
| 728 | 31096 | B | 737 | 1 | 1308 | |
| 729 | 31097 | B | 738 | 1 | 291 | |
| 730 | 31098 | C | 739 | 1 | 702 | |
| 731 | 31099 | B | 740 | 1 | 379 | |
| 732 | 31100 | B | 741 | 80 | 2747 | |
| 733 | 31101 | B | 742 | 1 | 1992 | |
| 734 | 31102 | B | 743 | 293 | 1296 | |
| 735 | 31103 | C | 744 | 769 | 1017 | |
| 736 | 31104 | C | 745 | 166 | 294 | |
| 737 | 31105 | B | 746 | 928 | 1483 | |
| 738 | 31106 | B | 747 | 247 | 375 | |
| 739 | 31107 | C | 748 | 47 | 582 | |
| 740 | 31108 | B | 749 | 47 | 388 | |
| 741 | 31109 | B | 750 | 53 | 458 | |
| 742 | 31110 | C | 751 | 32 | 277 | |
| 743 | 31111 | B | 752 | 1 | 1641 | |
| 744 | 31112 | C | 753 | 1 | 483 | |
| 745 | 31113 | B | 754 | 1 | 1518 | |
| 746 | 31114 | B | 755 | 1 | 321 | |
| 747 | 31115 | C | 756 | 604 | 779 | |
| 748 | 31116 | B | 757 | 695 | 967 | |
| 749 | 31117 | B | 758 | 1 | 768 | |
| 750 | 31118 | B | 759 | 101 | 531 | |
| 751 | 31119 | B | 760 | 1 | 1014 | |
| 752 | 31120 | C | 761 | 424 | 564 | |
| 753 | 31121 | B | 762 | 1 | 333 | |
| 754 | 31122 | B | 763 | 15 | 165 | |
| 755 | 31123 | B | 764 | 1 | 555 | |
| 756 | 31124 | B | 765 | 344 | 476 | |
| 757 | 31125 | B | 766 | 1 | 648 | |
| 758 | 31126 | B | 767 | 1 | 981 | |
| 759 | 31127 | C | 768 | 22 | 162 | |
| 760 | 31128 | B | 769 | 1 | 225 | |
| 761 | 31129 | B | 770 | 232 | 1671 | |
| 762 | 31130 | B | 771 | 166 | 504 | |
| 763 | 31131 | B | 772 | 473 | 1694 | |
| 764 | 31132 | C | 773 | 232 | 414 | |
| 765 | 31133 | C | 774 | 374 | 463 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 766 | 31134 | B | 775 | 1 | 1128 | |
| 767 | 31135 | B | 776 | 337 | 1284 | |
| 768 | 31136 | C | 777 | 25 | 282 | |
| 769 | 31137 | C | 778 | 4 | 63 | |
| 770 | 31138 | C | 779 | 496 | 1041 | |
| 771 | 31139 | C | 780 | 234 | 365 | |
| 772 | 31140 | B | 781 | 1 | 669 | |
| 773 | 31141 | B | 782 | 228 | 305 | |
| 774 | 31142 | B | 783 | 102 | 755 | |
| 775 | 31143 | B | 784 | 1 | 465 | |
| 776 | 31144 | B | 785 | 45 | 336 | |
| 777 | 31145 | C | 786 | 220 | 366 | |
| 778 | 31146 | B | 787 | 332 | 456 | |
| 779 | 31147 | B | 788 | 169 | 450 | |
| 780 | 31148 | B | 789 | 1 | 1173 | |
| 781 | 31149 | B | 790 | 36 | 355 | |
| 782 | 31150 | C | 791 | 354 | 482 | |
| 783 | 31151 | C | 792 | 328 | 708 | |
| 784 | 31152 | B | 793 | 1 | 829 | |
| 785 | 31153 | B | 794 | 14 | 182 | |
| 786 | 31154 | B | 795 | 307 | 1412 | |
| 787 | 31155 | C | 796 | 3 | 332 | |
| 788 | 31156 | B | 797 | 57 | 704 | |
| 789 | 31157 | B | 798 | 1 | 2406 | |
| 790 | 31158 | C | 799 | 1 | 759 | |
| 791 | 31159 | B | 800 | 1 | 351 | |
| 792 | 31160 | B | 801 | 142 | 272 | |
| 793 | 31161 | B | 802 | 34 | 2951 | |
| 794 | 31162 | B | 803 | 92 | 994 | |
| 795 | 31163 | B | 804 | 115 | 1746 | |
| 796 | 31164 | C | 805 | 292 | 408 | |
| 797 | 31165 | B | 806 | 1 | 880 | |
| 798 | 31166 | C | 807 | 156 | 329 | |
| 799 | 31167 | C | 808 | 119 | 328 | |
| 800 | 31168 | C | 809 | 1 | 492 | |
| 801 | 31169 | B | 810 | 1 | 516 | |
| 802 | 31170 | B | 811 | 1 | 624 | |
| 803 | 31171 | B | 812 | 24 | 1868 | |
| 804 | 31172 | C | 813 | 164 | 208 | |
| 805 | 31173 | C | 814 | 91 | 249 | |
| 806 | 31174 | B | 815 | 1 | 1059 | |
| 807 | 31175 | C | 816 | 80 | 106 | |
| 808 | 31176 | C | 817 | 283 | 408 | |
| 809 | 31177 | C | 818 | 1 | 357 | |
| 810 | 31178 | C | 819 | 1 | 909 | |
| 811 | 31179 | B | 820 | 26 | 71 | |
| 812 | 31180 | B | 821 | 1 | 714 | |
| 813 | 31181 | B | 822 | 1 | 678 | |
| 814 | 31182 | B | 823 | 1 | 675 | |
| 815 | 31183 | B | 824 | 24 | 1046 | |
| 816 | 31184 | B | 825 | 1 | 933 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 817 | 31185 | B | 826 | 1 | 363 | |
| 818 | 31186 | B | 827 | 112 | 1655 | |
| 819 | 31187 | B | 828 | 1 | 417 | |
| 820 | 31188 | B | 829 | 88 | 1485 | |
| 821 | 31189 | C | 830 | 1 | 411 | |
| 822 | 31190 | B | 831 | 114 | 277 | |
| 823 | 31191 | C | 832 | 671 | 1039 | |
| 824 | 31192 | B | 833 | 63 | 342 | |
| 825 | 31193 | B | 834 | 3530 | 4798 | |
| 826 | 31194 | B | 835 | 1 | 333 | |
| 827 | 31195 | B | 836 | 1 | 831 | |
| 828 | 31196 | B | 837 | 1 | 2514 | |
| 829 | 31197 | B | 838 | 98 | 250 | |
| 830 | 31198 | B | 839 | 1 | 5247 | |
| 831 | 31199 | B | 840 | 1 | 531 | |
| 832 | 31200 | B | 841 | 167 | 466 | |
| 833 | 31201 | B | 842 | 160 | 417 | |
| 834 | 31202 | B | 843 | 215 | 380 | |
| 835 | 31203 | B | 844 | 706 | 1262 | |
| 836 | 31204 | B | 845 | 41 | 368 | |
| 837 | 31205 | C | 846 | 252 | 578 | |
| 838 | 31206 | C | 847 | 18 | 380 | |
| 839 | 31207 | C | 848 | 14 | 349 | |
| 840 | 31208 | B | 849 | 1 | 1176 | |
| 841 | 31209 | B | 850 | 244 | 1174 | |
| 842 | 31210 | C | 851 | 27 | 146 | |
| 843 | 31211 | B | 852 | 217 | 1866 | |
| 844 | 31212 | B | 853 | 98 | 242 | |
| 845 | 31213 | B | 854 | 52 | 2112 | |
| 846 | 31214 | B | 855 | 98 | 242 | |
| 847 | 31215 | C | 856 | 237 | 518 | |
| 848 | 31216 | C | 857 | 1 | 528 | |
| 849 | 31217 | C | 858 | 213 | 365 | |
| 850 | 31218 | B | 859 | 86 | 478 | |
| 851 | 31219 | B | 860 | 1 | 903 | |
| 852 | 31220 | B | 861 | 191 | 539 | |
| 853 | 31221 | C | 862 | 283 | 480 | |
| 854 | 31222 | B | 863 | 248 | 738 | |
| 855 | 31223 | B | 864 | 7 | 1602 | |
| 856 | 31224 | B | 865 | 113 | 375 | |
| 857 | 31225 | B | 866 | 50 | 435 | |
| 858 | 31226 | B | 867 | 50 | 646 | |
| 859 | 31227 | B | 868 | 1 | 2292 | |
| 860 | 31228 | B | 869 | 1 | 2385 | |
| 861 | 31229 | B | 870 | 184 | 852 | |
| 862 | 31230 | B | 871 | 1 | 408 | |
| 863 | 31231 | B | 872 | 218 | 484 | |
| 864 | 31232 | B | 873 | 90 | 588 | |
| 865 | 31233 | B | 874 | 445 | 625 | |
| 866 | 31234 | B | 875 | 138 | 618 | |
| 867 | 31235 | B | 876 | 1 | 753 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Met hod | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 868 | 31236 | B | 877 | 1 | 489 | |
| 869 | 31237 | B | 878 | 113 | 366 | |
| 870 | 31238 | C | 879 | 271 | 489 | |
| 871 | 31239 | B | 880 | 918 | 3257 | |
| 872 | 31240 | B | 881 | 185 | 631 | |
| 873 | 31241 | C | 882 | 3 | 194 | |
| 874 | 31242 | B | 883 | 80 | 3219 | |
| 875 | 31243 | B | 884 | 213 | 1835 | |
| 876 | 31244 | C | 885 | 132 | 224 | |
| 877 | 31245 | B | 886 | 1 | 741 | |
| 878 | 31246 | C | 887 | 132 | 224 | |
| 879 | 31247 | B | 888 | 1 | 1281 | |
| 880 | 31248 | B | 889 | 125 | 1910 | |
| 881 | 31249 | B | 890 | 1 | 1449 | |
| 882 | 31250 | B | 891 | 284 | 696 | |
| 883 | 31251 | B | 892 | 139 | 390 | |
| 884 | 31252 | B | 893 | 1 | 1308 | |
| 885 | 31253 | B | 894 | 1 | 594 | |
| 886 | 31254 | B | 895 | 1 | 678 | |
| 887 | 31255 | B | 896 | 19 | 240 | |
| 888 | 31256 | B | 897 | 47 | 330 | |
| 889 | 31257 | B | 898 | 1 | 388 | |
| 890 | 31258 | B | 899 | 52 | 564 | |
| 891 | 31259 | C | 900 | 310 | 672 | |
| 892 | 31260 | B | 901 | 1 | 1338 | |
| 893 | 31261 | C | 902 | 77 | 214 | |
| 894 | 31262 | C | 903 | 213 | 467 | |
| 895 | 31263 | C | 904 | 202 | 426 | |
| 896 | 31264 | B | 905 | 68 | 567 | |
| 897 | 31265 | C | 906 | 32 | 205 | |
| 898 | 31266 | C | 907 | 513 | 701 | |
| 899 | 31267 | B | 908 | 1 | 1083 | |
| 900 | 31268 | B | 909 | 787 | 1633 | |
| 901 | 31269 | C | 910 | 40 | 288 | |
| 902 | 31270 | B | 911 | 178 | 330 | |
| 903 | 31271 | B | 912 | 129 | 520 | |
| 904 | 31272 | B | 913 | 2267 | 2626 | |
| 905 | 31273 | C | 914 | 34 | 87 | |
| 906 | 31274 | B | 915 | 23 | 610 | |
| 907 | 31275 | B | 916 | 1 | 1011 | |
| 908 | 31276 | B | 917 | 1 | 156 | |
| 909 | 31277 | B | 918 | 1 | 754 | |
| 910 | 31278 | B | 919 | 1 | 679 | |
| 911 | 31279 | B | 920 | 149 | 761 | |
| 912 | 31280 | B | 921 | 38 | 1175 | |
| 913 | 31281 | C | 922 | 542 | 724 | |
| 914 | 31282 | B | 923 | 31 | 283 | |
| 915 | 31283 | B | 924 | 21 | 341 | |
| 916 | 31284 | B | 925 | 199 | 361 | |
| 917 | 31285 | B | 926 | 293 | 427 | |
| 918 | 31286 | B | 927 | 56 | 145 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Method | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 919 | 31287 | B | 928 | 21 | 341 | |
| 920 | 31288 | B | 929 | 199 | 361 | |
| 921 | 31289 | B | 930 | 293 | 427 | |
| 922 | 31290 | B | 931 | 305 | 465 | |
| 923 | 31291 | B | 932 | 280 | 457 | |
| 924 | 31292 | C | 933 | 45 | 562 | |
| 925 | 31293 | B | 934 | 130 | 618 | |
| 926 | 31294 | B | 935 | 418 | 1620 | |
| 927 | 31295 | B | 936 | 115 | 252 | |
| 928 | 31296 | B | 937 | 1 | 573 | |
| 929 | 31297 | B | 938 | 1 | 2661 | |
| 930 | 31298 | B | 939 | 1 | 1345 | |
| 931 | 31299 | C | 940 | 747 | 1220 | |
| 932 | 31300 | C | 941 | 249 | 429 | |
| 933 | 31301 | B | 942 | 1 | 363 | |
| 934 | 31302 | C | 943 | 390 | 589 | |
| 935 | 31303 | B | 944 | 437 | 1553 | |
| 936 | 31304 | B | 945 | 1 | 1521 | |
| 937 | 31305 | C | 946 | 84 | 347 | |
| 938 | 31306 | B | 949 | 80 | 315 | |
| 939 | 31307 | B | 950 | 1 | 537 | |
| 940 | 31308 | C | 951 | 181 | 330 | |
| 941 | 31309 | C | 952 | 55 | 123 | |
| 942 | 31310 | C | 953 | 52 | 195 | |
| 943 | 31311 | C | 954 | 55 | 123 | |
| 944 | 31312 | B | 955 | 336 | 648 | |
| 945 | 31313 | B | 956 | 1 | 894 | |
| 946 | 31314 | B | 957 | 239 | 1008 | |
| 947 | 31315 | B | 958 | 126 | 308 | |
| 948 | 31316 | B | 959 | 1 | 747 | |
| 949 | 31317 | B | 960 | 101 | 351 | |
| 950 | 31318 | B | 961 | 179 | 1161 | |
| 951 | 31319 | B | 962 | 1 | 138 | |
| 952 | 31320 | B | 963 | 8 | 791 | |
| 953 | 31321 | C | 964 | 218 | 358 | |
| 954 | 31322 | C | 965 | 155 | 454 | |
| 955 | 31323 | C | 966 | 124 | 303 | |
| 956 | 31324 | C | 967 | 1 | 246 | |
| 957 | 31325 | B | 968 | 208 | 364 | |
| 958 | 31326 | C | 969 | 95 | 256 | |
| 959 | 31327 | C | 970 | 312 | 467 | |
| 960 | 31328 | B | 971 | 92 | 424 | |
| 961 | 31329 | B | 972 | 88 | 147 | |
| 962 | 31330 | C | 973 | 434 | 775 | |
| 963 | 31331 | B | 974 | 26 | 1781 | |
| 964 | 31332 | C | 975 | 363 | 692 | |
| 965 | 31333 | B | 976 | 201 | 563 | |
| 966 | 31334 | B | 977 | 348 | 687 | |
| 967 | 31335 | C | 978 | 529 | 660 | |
| 968 | 31336 | C | 979 | 418 | 738 | |
| 969 | 31337 | C | 980 | 25 | 177 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Method | SEQ ID NO: in USSN 09/540,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 970 | 31338 | B | 981 | 308 | 388 | |
| 971 | 31339 | C | 982 | 230 | 580 | |
| 972 | 31340 | B | 983 | 101 | 342 | |
| 973 | 31341 | B | 984 | 1 | 2341 | |
| 974 | 31342 | C | 985 | 1 | 642 | |
| 975 | 31343 | B | 986 | 1 | 1173 | |
| 976 | 31344 | B | 987 | 39 | 6743 | |
| 977 | 31345 | B | 988 | 1 | 516 | |
| 978 | 31346 | B | 989 | 1 | 756 | |
| 979 | 31347 | B | 990 | 1 | 912 | |
| 980 | 31348 | B | 991 | 310 | 441 | |
| 981 | 31349 | C | 992 | 58 | 300 | |
| 982 | 31350 | B | 993 | 80 | 1344 | |
| 983 | 31351 | C | 994 | 325 | 414 | |
| 984 | 31352 | B | 995 | 80 | 1582 | |
| 985 | 31353 | C | 996 | 143 | 499 | |
| 986 | 31354 | B | 997 | 173 | 375 | |
| 987 | 31355 | C | 998 | 126 | 268 | |
| 988 | 31356 | B | 999 | 1 | 762 | |
| 989 | 31357 | B | 1000 | 1 | 642 | |
| 990 | 31358 | B | 1001 | 1 | 1980 | |
| 991 | 31359 | B | 1002 | 67 | 456 | |
| 992 | 31360 | B | 1003 | 48 | 335 | |
| 993 | 31361 | B | 1004 | 1 | 1251 | |
| 994 | 31362 | B | 1005 | 1 | 642 | |
| 995 | 31363 | B | 1006 | 1 | 570 | |
| 996 | 31364 | C | 1007 | 1 | 687 | |
| 997 | 31365 | B | 1008 | 1 | 5450 | |
| 998 | 31366 | B | 1009 | 586 | 852 | |
| 999 | 31367 | B | 1010 | 299 | 530 | |
| 1000 | 31368 | B | 1011 | 1 | 1659 | |
| 1001 | 31369 | B | 1012 | 2 | 550 | |
| 1002 | 31370 | C | 1013 | 2 | 97 | |
| 1003 | 31371 | B | 1014 | 1114 | 1476 | |
| 1004 | 31372 | B | 1015 | 22 | 822 | |
| 1005 | 31373 | C | 1016 | 646 | 903 | |
| 1006 | 31374 | C | 1017 | 1 | 351 | |
| 1007 | 31375 | B | 1018 | 226 | 1284 | |
| 1008 | 31376 | B | 1019 | 138 | 997 | |
| 1009 | 31377 | B | 1020 | 341 | 527 | |
| 1010 | 31378 | B | 1021 | 157 | 1415 | |
| 1011 | 31379 | B | 1022 | 55 | 211 | |
| 1012 | 31380 | B | 1023 | 55 | 211 | |
| 1013 | 31381 | C | 1024 | 18 | 197 | |
| 1014 | 31382 | B | 1025 | 1 | 876 | |
| 1015 | 31383 | B | 1026 | 276 | 487 | |
| 1016 | 31384 | B | 1027 | 1 | 294 | |
| 1017 | 31385 | B | 1028 | 273 | 377 | |
| 1018 | 31386 | B | 1029 | 1 | 936 | |
| 1019 | 31387 | B | 1030 | 1 | 1158 | |
| 1020 | 31388 | C | 1031 | 104 | 283 | |

| SEQ ID NO: | SEQ ID NO: of peptide sequence | Method | SEQ ID NO: in USSN 09/840,217 | Nucleotide location of first codon for peptide sequence | Nucleotide location of last codon for last amino acid of peptide sequence | Amino acid sequence ( X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion) |
|---|---|---|---|---|---|---|
| 1021 | 31389 | B | 1032 | 1 | 720 | |
| 1022 | 31390 | B | 1033 | 1 | 219 | |
| 1023 | 31391 | B | 1034 | 1 | 170 | |
| 1024 | 31392 | B | 1035 | 300 | 831 | |
| 1025 | 31393 | C | 1036 | 1 | 456 | |
| 1026 | 31394 | B | 1037 | 1 | 1149 | |
| 1027 | 31395 | B | 1038 | 1 | 627 | |
| 1028 | 31396 | B | 1039 | 161 | 375 | |
| 1029 | 31397 | B | 1040 | 1 | 360 | |
| 1030 | 31398 | B | 1041 | 1 | 549 | |
| 1031 | 31399 | B | 1042 | 1 | 384 | |
| 1032 | 31400 | B | 1046 | 1 | 675 | |
| 1033 | 31401 | C | 1047 | 379 | 675 | |
| 1034 | 31402 | B | 1048 | 166 | 388 | |
| 1035 | 31403 | B | 1049 | 26 | 66 | |
| 1036 | 31404 | B | 1050 | 1 | 897 | |
| 1037 | 31405 | B | 1051 | 30 | 1359 | |
| 1038 | 31406 | B | 1052 | 1 | 990 | |
| 1039 | 31407 | B | 1053 | 52 | 1507 | |
| 1040 | 31408 | C | 1054 | 66 | 290 | |
| 1041 | 31409 | B | 1055 | 158 | 2072 | |
| 1042 | 31410 | B | 1056 | 1 | 654 | |
| 1043 | 31411 | B | 1057 | 51 | 1143 | |
| 1044 | 31412 | C | 1058 | 66 | 290 | |
| 1045 | 31413 | B | 1059 | 547 | 1510 | |
| 1046 | 31414 | B | 1060 | 1 | 1499 | |
| 1047 | 31415 | B | 1061 | 1 | 3347 | |
| 1048 | 31416 | C | 1062 | 116 | 235 | |
| 1049 | 31417 | B | 1063 | 1 | 1185 | |
| 1050 | 31418 | C | 1064 | 221 | 823 | |
| 1051 | 31419 | B | 1065 | 235 | 359 | |
| 1052 | 31420 | C | 1066 | 1 | 360 | |
| 1053 | 31421 | B | 1067 | 49 | 386 | |
| 1054 | 31422 | C | 1068 | 63 | 383 | |
| 1055 | 31423 | B | 1069 | 60 | 213 | |
| 1056 | 31424 | B | 1070 | 1 | 919 | |
| 1057 | 31425 | B | 1071 | 294 | 557 | |
| 1058 | 31426 | B | 1072 | 1 | 486 | |
| 1059 | 31427 | B | 1073 | 1 | 450 | |
| 1060 | 31428 | C | 1074 | 28 | 207 | |
| 1061 | 31429 | B | 1075 | 1 | 585 | |
| 1062 | 31430 | B | 1076 | 60 | 213 | |
| 1063 | 31431 | B | 1077 | 18 | 457 | |
| 1064 | 31432 | B | 1078 | 112 | 177 | |
| 1065 | 31433 | C | 1079 | 1 | 375 | |
| 1066 | 31434 | B | 1080 | 39 | 91 | |
| 1067 | 31435 | B | 1081 | 91 | 237 | |
| 1068 | 31436 | B | 1082 | 255 | 376 | |
| 1069 | 31437 | B | 1083 | 18 | 431 | |
| 1070 | 31438 | B | 1084 | 98 | 552 | |
| 1071 | 31439 | B | 1085 | 1679 | 1964 | |